Masten.Scott

From: Steve_Hentges@plastics.org
Sent: Thursday, July 26, 2001 3:06 PM
To: masten@niehs.nih.gov
Subject: Supplementary Information for July 25, 2001 FR Notice

BPA Final Report.doc

July 26, 2001

Scott Masten
Office of Chemical Nomination and Selection
NIEHS/NTP
PO Box 12233
Research Triangle Park, NC 27709

Dear Scott:

As we discussed by phone, I am writing to provide supplementary information that may be relevant to one of the chemicals recently nominated for testing by NTP (Federal Register, Volume 66, Number 143, Page 38717, July 25, 2001).

The chemical of interest is Diphenolic Acid (CAS 126-00-1), which was nominated by the National Cancer Institute (NCI) for genotoxicity and subchronic toxicity testing. Part of the stated rationale for nomination of this chemical is that it is structurally related to Bisphenol A. Although not explicitly stated in the Federal Register notice, NCI is presumably concerned with the potential carcinogenicity of Diphenolic Acid. Since the structural relationship with Bisphenol A was cited, it can also be inferred that NCI may consider that carcinogenic potential for Bisphenol A has been established.

We have no specific knowledge of Diphenolic Acid and can offer no comments on whether Bisphenol A is a suitable structural surrogate for assessment of the potential carcinogenicity of Diphenolic Acid. However, we do have extensive information on the toxicity of Bisphenol A including it's potential carcinogenicity. Attached is a comprehensive assessment on the carcinogenic potential of Bisphenol A, which has very recently been completed. The conclusion of this study, based on a weight-of-evidence assessment of a large body of relevant data, is that Bisphenol A is not carcinogenic. This assessment will be submitted for publication in a peer reviewed journal in the very near future.

I also would like to direct your attention to a comprehensive risk assessment on Bisphenol A that is being conducted on behalf of the European Union under Council Regulation (EEC) 793-93. Since the risk assessment is in near-final form but not yet complete, I have not sent the entire draft document (May 2001). However, the draft conclusions on carcinogenicity and mutagenicity of Bisphenol A, reproduced below, are consistent with the conclusion of the attached assessment.

European Risk Assessment Draft Conclusions on Bisphenol A

Carcinogenicity: "Taking into account all of the animal data available the evidence suggests that bisphenol-A does not have carcinogenic potential."

Mutagenicity: "Considering all of the available genotoxicity data, and the absence of significant tumour findings in animal carcinogenicity studies..., it does not appear that bisphenol-A has significant mutagenic potential in vivo."

Since testing on Diphenolic Acid was not recommended by NTP, it may be appropriate to forward the information in this note to NCI, who nominated the chemical for testing and presumably have a concern regarding potential carcinogenicity.

Please let me know if you would like a copy of the published version of the attached assessment, or the European risk assessment of Bisphenol A, when these documents become available. If you have any questions or need additional information, please feel free to contact me.

Regards,
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(See attached file: BPA Final Report.doc)

(Note the attached report is a read-only document. Simply click on the "Read Only" button that will appear when the document is opened to access the full document.)

An Evaluation of the Carcinogenic Potential of Bisphenol A

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February 21, 2001

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AN EVALUATION OF THE CARCINOGENIC POTENTIAL OF BISPHENOL A

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AN EVALUATION OF THE CARCINOGENIC POTENTIAL OF BISPHENOL A

EXECUTIVE SUMMARY

Bisphenol A (BPA), a monomer component of polycarbonate plastics and epoxy resins that are used in numerous consumer products, was evaluated for carcinogenic potential using internationally accepted criteria. It was concluded that BPA is not carcinogenic.

BPA has been tested for carcinogenicity by the National Toxicology Program (NTP) in 2-year feeding studies using F344 rats and B6C3F$_1$ mice. The overall results of these studies indicated that BPA lacks carcinogenic potential.

In the NTP rat study, there was no conclusive evidence of a carcinogenic effect of BPA. A higher incidence of leukemias was reported in high-dose males compared to the control group. However, this response was not significant when assessed using a life table analysis which adjusts for intercurrent mortality. Furthermore, the leukemia incidences were well within the range of historical control data for this strain. The incidence of leukemias in female rats was not statistically significant under any method of analysis. These results indicate that the incidence of leukemias was unlikely to be related to BPA treatment. The incidence of one other commonly occurring neoplasm of male rats, interstitial-cell (Leydig) tumors of the testes, was reportedly higher in both the low- and high-dose groups compared to the control group. However, this response was not considered treatment-related since interstitial-cell tumors occur at a very high incidence rate in aging F344 male rats, and the treatment group incident rates were within the historical control levels for the testing facility. The slightly longer survival of the treated male rats may also have influenced the incidence rate for this tumor type since this tumor appears late in the life of male F344 rats.

Likewise, there was no evidence of carcinogenic effects of BPA in the NTP mouse study. The incidence of leukemias and lymphomas in the low-dose male mice was reported to be significantly higher compared to controls. This response was not considered to be treatment-related as there was no dose-response (*i.e.*, no effect at the high-dose level) and no effect in females. Also, the types of hematopoietic tumors generally were typical of those occurring spontaneously in aging mice and the incidence rate was within historical control data for malignant lymphoma for this strain. The incidence of multinucleated giant hepatocytes was significantly higher in low- and high-dose male mice, compared to controls. However, this

change is of a non-neoplastic nature and was not associated with a higher incidence of liver tumors.

Furthermore, the results of metabolism studies provide no evidence to indicate formation of potentially reactive (carcinogenic) metabolites. Rather, these studies show that orally ingested BPA, following absorption, is metabolized by glucuronidation, a detoxification mechanism, and excreted as the glucuronide conjugate or in unchanged form in the feces and urine within 24 hours.

Oral toxicology studies in rats, mice and dogs indicate that BPA is reasonably well tolerated, even at high doses, and is associated with few consistent toxic effects. BPA has been reported to exhibit potential endocrine disrupting activity based primarily on *in vitro* results; however, results *in vitro* have not been unequivocally confirmed *in vivo*. The perturbation of endocrine function in either males or females may have adverse effects on endocrine-related tissues and functions, including reproduction. BPA has been shown to bind to estrogen receptors *in vitro*, albeit with much lower affinity than estradiol. Weak estrogen-like activity has been reported in certain rodent studies, possibly dependent upon the experimental system employed; however, adverse effects on reproductive function and development have not been established at doses that are not maternally toxic. The NTP 2-year carcinogenicity studies with BPA have not provided an indication of tumorigenic activity, proliferative cell growth, or otherwise adverse effects on endocrine-associated tissues. Thus, *in vitro* and *in vivo* assays demonstrating estrogen-like activity are of questionable relevance to assessing the carcinogenicity and reproductive toxicity of BPA, given the absence of endocrine-related tumors or any other adverse effects on reproductive function reported in well-conducted animal studies.

The overall weight of evidence from mutagenicity/genotoxicity studies indicates that BPA would not be genotoxic to humans. The lack of genotoxic activity in standard short-term assays is consistent with the lack of carcinogenic activity observed in the 2-year rat and mouse studies conducted by the NTP. BPA produced predominantly negative results in standard genotoxicity assays including bacterial reverse mutation tests (Ames tests), mammalian cell gene mutation and chromosome aberration tests, sister chromatid exchange (SCE) assays and the *in vivo* mouse micronucleus assay. The few positive *in vitro* findings were generally observed in non-standard tests including cell-free systems investigating aneuploidogenic potential and effects on microtubule assembly. It has been suggested that inhibition of microtubule assembly, which can lead to aneuploidy, may be associated with quinone forms, which can bind to the thiol groups of tubulin. *In vitro* DNA adduct formation by BPA also has been attributed to bisphenol-o-quinone. However, metabolism studies in rats, as well as results from isolated rat hepatocytes (*in vitro* metabolism), have demonstrated that absorbed BPA would be rapidly converted to bisphenol A-glucuronide, and not the quinone derivative. The lack of aneugenic effects in the *in vivo* mouse micronucleus assay supports a metabolism difference between *in vitro* and *in vivo* responses. Hydroxylation (phase I) resulting in quinone formation is generally a minor route for metabolism of phenolic compounds; however, with saturation of the glucuronidation (phase II) capacity, it is possible that some of the BPA may be converted to the quinone form. In an *in vivo* DNA adduct study in rats, the 2 major adducts were reported to match the adduct profile of DNA (*i.e.*, dGMP-biphenol-o-quinone) determined in an *in vitro* study. However, given the lack of mutation and clastogenicity observed in standard genotoxicity assays, the formation of rat hepatic DNA adducts at a high BPA doses appears to be of little relevance for human health considering the very low exposures that would be associated with use of products formulated with BPA.

The available data, including the results of metabolic studies, short-term assays for mutagenic/genotoxic activity, and the long-term toxicity and carcinogenicity studies conducted with rats and mice, were evaluated using a weight-of-evidence approach recommended by the International Agency for Research on Cancer (IARC) and the U.S. Environmental Protection Agency (U.S. EPA). It was concluded that BPA is not carcinogenic. The data underpinning this conclusion include:

< The results of the NTP studies that provide no substantive evidence to indicate that BPA is carcinogenic.

< The results of short-term tests of genetic toxicity which demonstrate that BPA is without genotoxic or mutagenic activity *in vivo*.

< The metabolic data which do not support the formation of potentially reactive intermediates and, moreover, which demonstrate that BPA is rapidly glucuronidated and excreted.

< The lack of any reports of association between exposure to BPA and human cancer.

< The low level of potential human exposure.

BISPHENOL A: AN EVALUATION OF CARCINOGENIC POTENTIAL

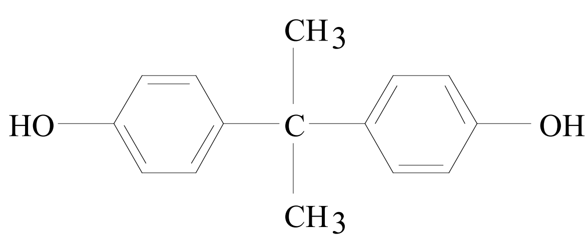
1.0 INTRODUCTION

Bisphenol A (BPA) is a monomer component of polycarbonate plastics and epoxy resins that are used in numerous consumer products. A major use of polycarbonate plastics is in food contact plastics. Recent articles have appeared which have implied that BPA may potentially be carcinogenic (Consumer Reports, 2000a,b). The present report presents an evaluation of the carcinogenic potential of BPA using internationally accepted criteria and a weight-of-evidence approach recommended by the International Agency for Research on Cancer (IARC) and the U.S. Environmental Protection Agency (U.S. EPA).

The report presents a review of potential human exposure (Section 2.0), discussions of the relevant studies of carcinogenic potential of BPA in humans and animals (Sections 3.0 and 4.0), and a review of other data, including the results of metabolic, genotoxicity, and reproductive toxicity studies pertinent to the overall evaluation of the carcinogenic potential of BPA (Section 5.0). In the evaluation section (Section 6.0), the available data are interpreted within a weight-of-evidence framework to characterize the carcinogenic potential of BPA. On the basis of this evaluation, BPA was concluded to be not carcinogenic.

2.0 EXPOSURE DATA

2.1 Chemical and Physical Data



$C_{15}H_{16}O_2$ Mol. wt: 228.28

Chem. Abstr. Services Reg. No. 80-05-7
Systematic name: 4,4'-(1-Methylethylidene)bisphenol

Synonyms:	80-05-7; bisferol A; bishpenol A; 2,2-bis-4'-hydroxyfenylpropan; 4,4'-isopropylidenediphenol; 2,2-bis(4-hydroxyphenyl)propane; 2,2-bis(p-hydroxyphenyl)propane; bis(4-hydroxyphenyl)dimethylmethane; bis(4-hydroxyphenyl)propane; 4,4'-bisphenol A; 4,4'-dihydroxydiphenyldimethylmethane; p,p'-dihydroxydiphenyldimethylmethane; 2,2-(4,4'-dihydroxydiphenyl)propane; 4,4'-dihydroxydiphenylpropane; p,p'-dihydroxydiphenylpropane; 4,4'-dihydroxydiphenyl-2,2-propane; 2,2-di(4-hydroxyphenyl)propane; beta-di-p-hydroxyphenylpropane; dimethyl bis(p-hydroxyphenyl)methane; dimethylmethylene-p-,p -diphenol; diphenylolpropane; 2,2-di(4-phenylol)propane; 4,4'-isopropylidenebisphenol; p,p -isopropylidenebisphenol; p,p -isopropylidenediphenol; NCI-C50635
Description:	crystals or flakes
Boiling-point:	220¡C at 4 mm Hg
Melting-point:	150-155¡C
Solubility:	practically insoluble in water (<1 mg/ml at 25¡C); soluble in aqueous alkaline solutions, alcohol, acetone; slightly soluble in carbon tetrachloride.

2.2 Production, Use and Human Exposure

The estimated production capacity of BPA in the United States has been reported to be 860 x 10^6 kg per year. Nearly two-thirds (63%) of BPA is used in the production of polycarbonate plastic resins, while approximately 27% is used to produce epoxy resins. The remaining 10% is used in other various resin applications or in flame retardants.

Polycarbonate plastics have numerous end use applications, such as food and drink containers, reusable bottles, compact discs, and components of electronic equipment and household appliances. Epoxy resins are used in a variety of adhesive and composite product applications, including use as coatings and linings for food cans, restorative dental sealants, electrical laminates, and adhesives.

Although used in a number of consumer end-products, including food-contact materials, human exposure to BPA is limited by the low concentrations of residual BPA in these polycarbonate and epoxy resins, the chemical stability of these resins, and the negligible migration of BPA to foods under typical conditions of use.

Howe and Borodinsky (1998) reported residual BPA concentrations ranging from 8,800 to 11,200 g/kg (ppt) in molded test samples of representative food-contact polycarbonate. Migration experiments with this polycarbonate in contact with simulants representative of aqueous, low alcohol, acidic and fatty food matrices did not detect BPA to a detection limit of 5 g BPA/kg food. Assuming a maximum migration of 5 g BPA/kg food, Howe and Borodinsky (1998), using United States Food and Drug Administration (FDA) dietary consumption equations, estimated consumer exposure to BPA through food contact polycarbonate use to be <0.75 g/person/day. Assuming a body weight of 60 kg, the maximum daily dose of BPA resulting from the use of polycarbonate plastics in food-contact applications would be <0.0125 g/kg body weight/day. Kawamura *et al.* (1998) similarly found low migration of BPA from a variety of food-contact houseware products. Included in the products analyzed were items recalled under Japanese Food Hygiene Law in violation of the 500 ppm (mg/kg) limit for total BPA, phenol, and p-*tert*-butylphenol. Migration experiments were conducted using food simulant solvents at various temperatures. Kawamura *et al.* (1998) reported the migration of BPA was greatest in n-heptane at 25¡C for 60 minutes and least in water and 4% acetic acid at 60¡C for 30 minutes. Boiling of the samples prior to migration testing decreased the amount of BPA detected, suggesting the migration of BPA was limited to residues at or near the food-contact surface. The maximum amount of BPA detected in extraction solvent from housewares containing >500 mg/kg BPA was <40 g/kg, indicating a low migration tendency. In non-recalled mugs, rice bowls, nursing bottles and a measuring cup, with residual BPA concentrations ranging from non-detected to 80 mg/kg, Kawamura *et al.* (1998) detected migratory BPA in only 2 samples. The maximum concentration of BPA detected was <5 g/kg.

Similarly, studies conducted with food cans lined with BPA-based epoxy resins demonstrate negligible migration. Brotons *et al.* (1995) analyzed a variety of canned vegetables and reported BPA concentrations ranging from non-detected to approximately 23 g/can. Subsequent migration studies with epoxy resin-coated cans using simulated food-contact solvents resulted in an average BPA concentration of 37 g/kg in the contained food simulant (Howe *et al.* , 1998). Howe *et al.* (1998), using U.S. FDA dietary exposure equations, estimated a maximum daily exposure of 0.105 g BPA/kg body weight/day through food contact with epoxy resin-coated cans. This level is comparable to maximum cumulative exposure estimates to BPA for adults and infants, as determined by the FDA (FDA, 1996). Based on a conservative exposure assessment, the FDA calculated that cumulative exposure to BPA from use in can enamels and polycarbonate-based polymers would not be expected to exceed 11 g/person/day for adults (~0.183 g/kg body weight/day, assuming a body weight of 60 kg) or 7 g/person/day for infants (~1.75 0181 g/kg body weight/day, assuming a body weight of 4 kg). The infant value is particularly conservative since the maximum concentration of BPA detected in infant formula, 6.6 g/kg, was used to calculate daily exposure. In the studies reviewed, the concentration of BPA detected in formula ranged from 0.05 to 6.6 g/kg with an average concentration of 2.5 g/kg.

The use of epoxy resins in restorative dental sealant composites has been reported as a potential source of BPA exposure. Exposure would presumably occur through the leaching of unreacted or otherwise residual BPA (Olea *et al.*, 1996; Pulgar *et al.*, 2000). Olea *et al.* (1996) reported apparent BPA concentrations ranging from approximately 3 to 30 mg/L in the saliva of 18 patients 1 hour after receiving 50 mg of dental sealant. The validity of these concentrations, however, has been questioned from an analytical perspective (Fung *et al.*, 2000). A subsequent study by Fung *et al.* (2000) reported substantially lower concentrations of approximately 6 to 106 g BPA/L in the saliva of 40 dental patients, 1 and 3 hours after receiving 8 or 32 mg sealant. Fung *et al.* (2000) were unable to detect BPA in any saliva samples taken at 24 hours following treatment. Furthermore, Fung *et al.* (2000) was unable to detect BPA in the serum of any patient sampled from 1 hour to 5-days post-sealant exposure. Thus, minimal concentrations of BPA may be detected in saliva immediately following restorative work, however, given the rapid elimination of BPA following absorption, plasma levels would be minute. An analysis of the 12 dental sealants approved by the American Dental Association indicated no detectable (<5 g/kg) concentrations of BPA in all sealants (ADA, 1998). Based on the preceding analysis, the potential exposure of consumers to BPA through the use of epoxy resins in dental sealants is not directly quantifiable; however, it appears of negligible consequence.

3.0 STUDIES OF CANCER IN HUMANS

No epidemiology studies of BPA were available.

4.0 STUDIES OF CANCER IN EXPERIMENTAL ANIMALS

The carcinogenic potential of BPA was evaluated by the National Toxicology Program in 2-year feeding studies using F344 rats and $B6C3F_1$ mice (NTP, 1982). Groups of 50 rats per sex were administered diets containing 0, 1,000, or 2,000 ppm of BPA. Groups of 50 male mice were administered 0, 1,000, or 5,000 ppm BPA, while females, also in groups of 50, were administered 0, 5,000, or 10,000 ppm. These dietary levels corresponded to intakes of approximately 0, 74, and 148 mg/kg/day and 0, 74, and 135 mg/kg/day in male and female rats, respectively, and of 0, 120, and 600 mg/kg/day and 0, 650, and 1,300 mg/kg/day in male and female mice, respectively. Clinical examinations for signs of toxicity were conducted twice daily for all animals and body weight measurements were taken every 2 weeks for the first 13 weeks and monthly thereafter. Thorough gross pathology and histological examinations were conducted on all animals, including those that died prior to scheduled sacrifice.

In rats, body weight gain and feed consumption were reduced in all treated groups of males and females compared to the corresponding controls. No other treatment-related clinical signs were observed. Survival of treated animals was not significantly different from controls. Among males, the number of animals surviving to the end of the study were 23/50 (46%), 30/50 (60%), and 27/50 (54%) for the control, low- and high-dose groups, respectively. Survival among females was 35/50 (70%), 35/50 (70%), and 37/50 (74%), for the control, low- and high-dose groups, respectively.

A higher incidence of leukemias was observed in high-dose male rats (23/50; 46%) compared to the control group (13/50; 26%). This response was statistically significant by a Cochran-Armitage test (P=0.021) but was not significant when assessed using a life table analysis, which adjusted for intercurrent mortality (high dose effect, P=0.141; dose-response trend, p=0.074). The high dose effect also was not statistically significant using the Fisher exact test (P=0.030), as the level of significance is above the value of p=0.025 required by the Bonferroni inequality criterion for an overall significance of p=0.05 when two dosed groups are compared with a common control group. Furthermore, the leukemia incidences were well within the range of historical control data for this strain (10 to 74%) (Haseman *et al.,* 1990, 1998). The incidence of leukemias in low-dose males (12/50; 24%) was not significantly different from controls. Similarly, in females, the incidence of this tumor (7/50, 13/50, and 12/50 for control, low and high dose females, respectively) was not statistically significant under any method of analyses.

The incidence of interstitial-cell tumors (Leydig cell tumors) in male rats was significantly higher in both the low (48/50; 96%) and high (46/49; 94%) dose groups compared to the control group (35/49; 71%). Although statistically significant, this response was not considered treatment-related since interstitial-cell tumors occur at a very high incidence rate in aging F344 male rats (>90%) (NTP, 1982), and the treatment group incident rates were within the historical control levels (mean of 88%) for the testing facility. Furthermore, the non-significant, but greater survival among treated male rats compared to controls may have influenced the incidence rate for this tumor type.

Fibroadenomas of the mammary gland in male rats were observed in greater proportions in the high-dose group (0/50, 0% in the controls; 0/50, 0% in the low-dose; and 4/50, 8% in the high-dose group), but were not statistically significant compared to the control group using the Fisher exact tests. This tumor type was not significantly increased in female rats (8/50, 8/50, 5/50 for control, low dose, and high dose, respectively).

Treatment of mice resulted in decreased mean body weights, in females and in high-dose males. Food consumption among all groups of treated mice was not significantly different from the respective controls. No other treatment-related clinical signs were observed. Survival for male and female mice in test groups was not significantly different from control groups. Survival rates were 42/49 (86%), 37/50 (74%), and 38/50 (76%) for control, low- and high-dose male mice, respectively. Among female mice, survival rates were 39/50 (78%), 37/48 (77%), and 41/48 (85%) for control, low- and high-dose groups, respectively.

Male mice in the low-dose group exhibited a marginally significant higher incidence of lymphomas and leukemias combined, however, there was no dose-response relationship (2/49, 4% for controls; 9/50, 18% for low-dose; 5/50, 10%, for high-dose), and this tumor type was not significantly higher in females (11/50, 8/48, 8/48, for control, low-dose, and high-dose groups, respectively). Also, the types of hematopoietic tumors that were observed generally were typical of those occurring spontaneously in aging mice, and the incidence rates were within the range of historical control data for malignant lymphoma (2 to 20%) for this species (Haseman *et al.,* 1998). The incidence of multinucleated giant hepatocytes, a non-neoplastic change, was significantly higher in low- (41/49, 84%) and high-dose male mice (41/50, 82%) compared to controls (1/49, 2%). However, these changes were not observed in female mice and were not associated with a higher incidence of liver tumors.

The NTP concluded that the level of evidence of carcinogenicity for BPA was equivocal for male rats, negative for female rats, and negative for both male and female mice. Overall, the results of this bioassay did not provide any convincing evidence to indicate that BPA was carcinogenic in F344 rats or B6C3F1 mice.

5.0 OTHER DATA RELEVANT TO AN EVALUATION OF CARCINOGENICITY AND ITS MECHANISMS

5.1 Absorption, Distribution, Metabolism and Excretion

There are a limited number of studies evaluating the absorption, distribution, metabolism, and elimination of BPA, however, in general, BPA is readily absorbed and distributed, is not bioaccumulated in tissues, and is rapidly eliminated within 24 hours of oral administration. The primary route of excretion is in the feces as unmetabolized BPA or, to a lesser extent, in the urine as a glucuronide conjugate. BPA and its metabolites may be more bioavailable following subcutaneous or intraperitoneal injection; however, these routes of administration are not of relevance to human exposure.

Knaak and Sullivan (1966) investigated the metabolism of BPA in male rats over 8 days following oral administration of approximately 800 mg BPA/kg body weight. Knaak and Sullivan (1966) reported that the majority of radioactivity was excreted within 24 to 48 hours, with the cumulative dose excreted reaching a plateau from Days 3 through 8. In 3 studies with 4 rats/study, Knaak and Sullivan (1966) reported a mean total recovery of 88% of administered dose over 8 days. An average of 28% was recovered from urine (32% of total recovered) and 56% from feces (64% of total recovered), with no radioactivity detected in the gastrointestinal tract or carcass of rats at termination following 8 days. The majority of radioactivity excreted in the urine was identified as a glucuronide-conjugate of BPA, with <1% excreted as parent BPA. In the feces, parent and hydroxylated BPA were identified as the primary metabolites, with unidentified metabolites accounting for approximately 30% of the radioactivity excreted.

More recently, Pottenger *et al.* (2000) confirmed the results of Knaak and Sullivan (1966) in studies using both male and female Fischer 344 rats. In addition to oral administration of BPA, Pottenger *et al.* (2000) evaluated metabolism following subcutaneous (*sc*) and intraperitoneal (*ip*) administration. Rats were administered a single dose of 10 or 100 mg ^{14}C-BPA/kg body weight by oral gavage, or *sc* or *ip* injection, and blood, urine, and feces collected over 7 days until sacrifice. An additional experiment was conducted with similar doses and routes of administration to compare plasma metabolites at specific pharmacokinetic time-points. Of the total radioactivity recovered, the feces represented the major route of elimination for all means of administered dose, in both males and females, and at both dose concentrations. Of the mean 88 to 98% of radioactivity recovered in males, 82 to 85% was recovered in the feces, 14 to 16% was recovered in the urine, and <1.3% was residual in the tissues at sacrifice. The mean recovery of radioactivity was slightly lower in female rats, with 84 to 85% of the administered dose recovered following *sc* administration, 86 to 88% with *ip*, and 96 to 97% with oral gavage. In females, 60 to 74% of the recovered radioactivity occurred in the feces, 24 to 38% was present in the urine, and <1.5% was residual in the tissues at sacrifice. The majority of radioactivity excreted in the feces, 86 to 93%, was identified as parent BPA for all routes of administration. The monoglucuronide conjugate of BPA was identified as the predominant urinary metabolite, comprising 57 to 68% of urinary radioactivity in males, and 70 to 87% of urinary radioactivity in females. Parent BPA represented 3 to 12% of radioactivity detected in the urine, with a monosulfate conjugate metabolite of BPA representing 2 to 7% of radioactivity. Pottenger *et al.* (2000) did not detect the hydroxylated BPA metabolite reported by Knaak and Sullivan (1966) and suggested the higher dose administered by Knaak and Sullivan (1966), in addition to the different strain of rat used, may have exceeded the metabolic capacity of the rats, and may have been responsible for this observation.

Pottenger *et al.* (2000) reported that the pharmacokinetics of BPA in the blood and of BPA-derived radioactivity in the plasma were dose-related, dependent on the route of administration, and varied slightly with sex. BPA that was administered orally was less bioavailable than BPA injected *sc* or *ip*. Blood concentrations of BPA were not quantifiable in males (limit of quantitation 0.01 mg/kg blood) following oral administration of 10 mg BPA/kg body weight, whereas females had a maximum blood concentration of 0.04 mg/kg at 15 minutes post-dosing. With a dose of 100 mg BPA/kg body weight, males had a maximum blood concentration of 0.22 mg/kg at 5 minutes post-dosing, whereas females had a maximum blood concentration of 2.29 mg/kg at 15 minutes. The time to non-quantifiable blood concentrations in rats administered oral doses of 10 or 100 mg BPA/kg body weight ranged from 5 to 45 minutes in males, and from 1 to 18 hours in females. Similar sex-related differences in blood concentrations of BPA were not observed for the *sc* and *ip* routes of administration. Plasma radioactivity was also quantitatively lower in orally dosed rats compared to those administered *sc* or *ip*. Maximum plasma radioactivity occurred 5 to 15 minutes post-dosing and was not quantifiable in both sexes beyond 72 hours post-dosing. Pottenger *et al.* (2000) attributed metabolic clearance *via* first-pass metabolism as the determining factor of oral bioavailability compared to limited intestinal absorption, as the length of time taken for radioactivity to appear in the feces was longer than the typical gastrointestinal transit time. Furthermore, Pottenger *et al.* (2000) reported the identities and relative proportions of BPA metabolites were dependent upon the route of administration. For example, the monoglucuronide conjugate accounted for 68 to 100% of plasma radioactivity in orally dosed rats, whereas parent BPA accounted for 27 to 51% and 65 to 76% of plasma radioactivity following *ip* and *sc* administration, respectively. Qualitatively, *ip* and *sc* administration contributed to unique metabolite profiles that were not consistent with that observed with oral administration. Based on these observations, Pottenger *et al.* (2000)

cautioned against using non-oral routes of administration to extrapolate the metabolic fate of orally consumed BPA.

Takahashi and Oishi (2000) orally administered 1,000 mg BPA/kg body weight to pregnant F344/DuCrj (Fischer) rats on Day 18 of gestation and collected maternal blood, liver and kidney tissues, and whole fetuses at 10 minutes to 48 hours following administration. Maximum maternal concentrations in the blood, liver and kidney occurred at 20 minutes post administration and were equivalent to 0.007, 0.083, and 0.017% of the administered dose per gram of respective tissue (14.7 mg/kg, 171 mg/kg, and 36.2 mg/kg, respectively). Concentrations at 10 hours post-administration were 20 to 100-fold lower than the maximum concentrations reported at 20 minutes. Calculated initial half-lives were 5.7, 10.7, and 14.7 minutes in blood, liver, and kidney, respectively. Concentrations were consistently highest in the liver and lowest in the blood. Takahashi and Oishi (2000) demonstrated BPA was readily transferred across the placenta as homogenates of whole fetuses contained BPA reflective of maternal concentrations, highest at 20 minutes post-administration (0.004% of administered dose per gram fetus, or 9.2 mg/kg), and 50 times lower at 10 hours post-administration. Calculated half-lives were slightly longer in whole fetus homogenates, with an initial half-life of 33 minutes; however, a direct comparison of the half-life of BPA in whole fetus homogenate with the calculated half-lives in individual maternal tissues is not appropriate. The concentration of BPA in whole fetus homogenates followed a similar pattern of elimination over time as the maternal samples. Takahashi and Oishi (2000) further suggested enterohepatic cycling contributed to an extended terminal half-life of BPA in rats.

The metabolism of BPA has been further evaluated *in vitro* with isolated hepatocytes and hepatic mitochondria from adult male F344/DuCrj rats (Nakagawa and Tayama, 2000). The incubation of hepatocytes with 0.25, 0.5, or 1.0 mM BPA for 3 hours resulted in a dose-dependent decrease in cell viability and intracellular ATP. Analyses of hepatocyte culture media over the duration of incubation indicated BPA concentrations exhibiting lesser toxicity *in vitro* were rapidly and extensively metabolized to the glucuronide conjugate. Metabolic studies *in vivo* (Knaak and Sullivan, 1966; Pottenger *et al.*, 2000) have demonstrated BPA-glucuronide conjugate to be the primary elimination metabolite in urine. With reduced cell viability, a greater percentage of unmetabolized parent BPA remained throughout the incubation period (Nakagawa and Tayama, 2000). For example, parent BPA was not detected following 2 hours of incubation with an initial BPA concentration of 0.25mM, whereas approximately 65% of parent BPA remained at the end of the incubation period at 0.5 mM, a concentration which decreased cell viability. Apart from the glucuronide conjugate, identified by enzymatic hydrolysis by _-glucuronidase, Nakagawa and Tayama (2000) did not identify other minor metabolic peaks from HPLC chromatograms.

Nakagawa and Tayama (2000) attributed the decrease in intracellular ATP observed in hepatocytes to a significant dose-dependent decrease in NAD^+- and FAD-linked respiratory activity and to inhibition of mitochondrial oxidative phosphorylation observed following BPA incubation with isolated hepatic mitochondria.

5.2 Toxic Effects

BPA was indicated to be of low acute oral toxicity based on studies in F344 rats and $B6C3F_1$ mice (NTP, 1982). The oral LD_{50} values determined were 4,100 mg/kg for male rats, 3,300 mg/kg for female rats, 5,200 mg/kg for male mice, and 4,100 mg/kg for female mice.

In addition to the carcinogenesis bioassays discussed previously, other feeding and oral gavage toxicity studies of BPA of up to 90 days duration have been completed in rats (NTP, 1982; Til *et al.*, 1978), mice (NTP, 1982, 1985a,b) and dogs (General Electric, 1976a,b).

In a two week feeding study in which groups of F344 rats (5/sex) were administered BPA at dietary levels of 0, 500, 1,000, 2,500, 5,000 or 10,000 ppm, mean body weight gain was decreased by 60% or greater in males receiving ‡ 2,500 ppm and by 40% or greater in females administered ‡ 5,000 ppm BPA (NTP, 1982). All animals survived for the duration of the exposure period. No other endpoints were investigated.

In groups of F344 rats (10/sex/group) fed BPA at dietary levels of 0, 250, 500, 1,000, 2,000, or 4,000 ppm for 13 weeks, BPA was reasonably well tolerated (NTP, 1982). No treatment-related deaths were observed, and other than decreased body weight gain at 1,000 ppm and above, the only possibly treatment-related effects observed, following a thorough necropsy examination, were hyaline masses in the bladder lumen of male rats and cecal enlargement in male and female rats. Histological examination of the cacal walls did not reveal any abnormalities, nor were there any clear signs of adverse effects associated with the kidney. Hyaline masses were not observed in rats in the 2-year cancer bioassay. This finding also has not been reported in other toxicology studies with BPA. Thus, this effect was likely a chance finding rather than treatment related.

In another feeding study with Wistar rats, groups of 15 rats per sex were fed diets containing 0, 100, 500 or 2,500 ppm BPA for 90 days (Til *et al.*, 1978). These dietary levels corresponded to 0, 7, 37, 182 mg/kg for male rats and 0, 7, 37, 185 mg/kg for female rats. Compared to controls, mean body weight gain was significantly reduced in high-dose males, and in mid- and high-dose females. Fasting blood glucose levels were significantly decreased in high-dose males, and in mid- and high-dose females. Significantly decreased creatine levels were observed in mid- and high-dose males, and significantly increased total white blood cells counts were observed in high-dose females. In high-dose groups, significantly increased organ weights, compared to controls, included the brain in both males and females, the testes in males, and the kidneys in females. At necropsy, cecal enlargement was observed in mid- and high-dose males, and high-dose females. Alopecia was observed in females in the low-, mid- and high-dose groups, and in 1 high-dose male. This effect has not been observed in any other study including the cancer bioassay and, thus, is likely a chance finding. No treatment-related histological changes were observed. The few effects observed including reduced body weight gain, increased organ weights, cecal enlargement, and clinical chemistry (blood glucose, creatine) and hematological (total white blood cell counts) changes are toxicologically insignificant in the absence of histological changes. Furthermore, the decrease in body weight gain may have been influenced by decreased palatability of the diet.

A series of dose-range finding and subchronic toxicity studies in mice were conducted by NTP (1982, 1985a,b). In one 10-day oral gavage study, groups of 8 female CD-1 mice were administered 0, 120, 250, 500, 1,000, 1,500, or 2,000 mg BPA/kg bodyweight in corn oil (NTP, 1985a). Mortalities were observed at the 2 highest doses groups (6/8 at 1,500 mg/kg and 7/8 at 2,000 mg/kg). Clinical signs of toxicity included rough coat starting at 120 mg/kg, lethargy and piloerection at 1,000 mg/kg, and ataxia, dyspnea, vocalization, hunched over appearance, and hypersensitivity at 1,500 and 2,000 mg/kg doses. Body weight gain and relative liver weights were unaffected. No detailed histological examinations were performed.

Similar results were obtained in a dietary study in CD-1 mice administered up to 5% BPA (NTP, 1985b). Groups of 8 mice per sex were fed diets containing 0, 0.31, 0.62, 1.25, 2.5, or 5% BPA (approximately 0, 372, 744, 1,500, and 3,000 mg/kg body weight in males and 0, 403, 806, 1,625, and 3,250 mg/kg body weight in females). Mortalities were observed at the high-dose level in both male and female rats (6/8 in both groups). Mean body weight gain was significantly reduced in males in the 2.5 and 5% dose groups and in the surviving females in the high-dose group. Clinical signs of toxicity included dehydration, dyspnea, lethargy, and ptosis at the 2 highest doses and piloerection, diarrhea, and moribundity at the highest dose. No other endpoints were assessed.

In a 90-day dietary study, groups of $B6C3F_1$ mice (10/sex/group) were fed diets containing 0, 5,000, 10,000, 15,000, 20,000 or 25,000 ppm BPA (NTP, 1982). These dose levels correspond approximately to 0, 600, 1,200, 1,800, 2,400 and 3,000 mg/kg body weight in males and 0, 650, 1,300, 1,950, 2,600, and 3,250 mg/kg body weight in females. No treatment related mortalities occurred. Mean body weight gain was reduced by 14% or greater in males starting at 15,000 ppm and by 17% or greater in females starting at the 5,000 ppm dose level, but was not dose-related. Multinucleated giant hepatocytes were observed in all treated males in a dose-response relationship, but not in female mice. This observation also was noted in male mice in the 2-year cancer bioassay. As discussed above (Section 4.0), this effect was not associated with increased incidences of liver tumors.

Two toxicology studies of BPA have been completed in dogs (General Electric, 1976a,b). In the first study (General Electric, 1976a), groups of 2 beagle dogs were fed diets containing 2,000, 4,000, 8,000, or 12,000 ppm BPA for a period of 2 weeks. These dietary levels corresponded to doses of 49, 88, 281, or 293 mg/kg body weight in males and 50, 137, 262, and 278 mg/kg body weight in females. No obvious signs of toxicity, changes in body weight gain or food consumption, compared to pre-treatment measurements, were observed. Slight focal mucosal congestion and hemorrhage of the gastrointestinal tract were observed at necropsy in some of the dogs.

In a 90-day dietary study by the same investigators, BPA was administered to beagle dogs (4/sex/group) at levels of 0, 1,000, 3,000, or 9,000 ppm (~ 0, 28, 74, or 261 mg/kg body weight in males and 0, 31, 87, or 286 mg/kg body weight in females) (General Electric, 1976b). BPA was well tolerated with no overt signs of toxicity. No treatment related effects were observed on body weight gain or food consumption, or on ophthalmology, hematology, biochemistry, or urinalysis parameters. Relative liver weights were increased 18% and 26% in high-dose males and females, respectively, compared to controls. Focal mucosal congestion and hemorrhage of gastrointestinal tract tissues, which were observed in some dogs in the 2-week range finding study, were not observed in this study.

The results of the oral toxicology studies in rats, mice and dogs indicate that BPA is associated with few consistent toxic effects, even at high doses.

5.3 Reproductive and Developmental Effects

Based primarily on *in vitro* results, BPA has been reported to exhibit potential endocrine disrupting activity, however, results *in vitro* have not been unequivocally confirmed *in vivo*. The perturbation of endocrine function in either males or females may have adverse effects on endocrine-related tissues and functions, including reproduction. BPA has been shown to bind to estrogen receptors *in vitro*, albeit with much lower affinity than estradiol. Weak estrogenic activity has been reported in certain rodent studies, possibly dependent upon the experimental system employed, however, adverse effects on reproduction function and the development of rats and mice have not been established at doses that are not maternally toxic. Furthermore, as noted in Section 4.0, chronic carcinogenicity and toxicity assays with BPA have not provided indication of tumorigenic activity, proliferative cell growth, or other adverse effects on endocrine-associated tissues.

In vitro assays demonstrate that BPA is capable of binding to estrogen receptors; however, with an affinity that is hundreds to thousands fold lower than 17-_-estradiol (Chun and Gorski, 2000; Laws *et al.*, 2000; Yoon *et al.*, 2000). In cell proliferation assays, BPA has been reported to stimulate proliferation in human breast MCF-7 cells at a potency of 3 to 5 orders of magnitude lower than estradiol (Brotons *et al.*, 1995; Villalobos *et al.*, 1995). In human endometrial ECC-1 cells, Bergeron *et al.* (1999) did not observe BPA-induced proliferation despite reported BPA binding to estrogen receptors and induction of progesterone receptor mRNA expression. In addition, *in vitro* and *in vivo* assays by Steinmetz *et al.* (1997) and Chun and Gorski (2000) have demonstrated BPA to increase prolactin secretion from rat pituitary cells, with a potency that is 5 to 6 orders of magnitude lower than estradiol. The data indicate that the estrogenic effects of BPA are dependent upon the experimental system employed and the physiological relevance of such reported observations is not clear (Zacharewski, 1998).

The endocrine disrupting activity of BPA was recently evaluated in female Long Evans rats by Laws *et al.* (2000), reportedly following proposed U.S. EPA chemical screening protocols (U.S. EPA, 1998), with some modifications. A 3-day uterotrophic assay in ovariectomized adult females administered 100 mg BPA/kg body weight/day by oral gavage indicated no increase in uterine weight when necropsied 6 hours after the last dose. In addition, ovariectomized adult females administered 100 mg BPA/kg body weight/day for 11 days by oral gavage exhibited no incidence of cornified vaginal epithelial cells, an indicator of estrus. In intact females, Laws *et al.* (2000) reported that 100 mg oral BPA/kg body weight/day for 25 days significantly reduced the mean number of 4- to 5-day cycles as compared to controls. However, the mean number of days of diestrus and estrus were not affected, and 7 of 15 females exhibited normal estrus cycling.

Steinmetz *et al.* (1998) reported hyperplasia, hypertrophy, and differentiation in the reproductive epithelial tissue of ovariectomized F344 rats following 3 days of exposure to *sc* implanted capsules releasing an estimated 0.3 mg crystalline BPA/kg body weight/day. This conclusion was based upon reported increased cell height of the luminal epithelium of the uterus, and increased thickness, keratinization, and sloughing of vaginal epithelial cells, compared with rats implanted with empty capsules. These effects, however, were not observed in similarly dosed ovariectomized SD rats. In addition, Steinmetz *et al.* (1998) evaluated the estrogenic activity of

BPA compared with estradiol through measurement of *c-fos* gene expression. Steinmetz *et al.* (1998) reported that a single *ip* injection of 50 mg BPA/kg body weight to ovariectomized F344 female rats induced *c-fos* mRNA expression, similar to that induced by 10 g estradiol/kg body weight, in the luminal epithelium when measured at 2 hours post-dosing in the uterus, and when measured at 2 and 6 hours post-dosing in the vagina. The induction of *c-fos* expression in SD rats dosed with BPA was not investigated. Thus, it is not known whether the observed *c-fos* induction with 50 mg BPA/kg body weight *ip* is independent of the results of the 3-day *sc* implantation study in both F344 and SD rats. Neither the proliferative changes in reproductive tissues in the 3-day *sc* release study, nor the increase in mRNA expression following a single *ip* dose, can be interpreted as relevant to the assessment of the carcinogenic potential of BPA. These observations are not supported by the chronic oral carcinogenicity assays conducted with higher doses that demonstrated an absence of carcinogenic potential for BPA (see Section 4.0).

In prepubertal females administered 100, 200, and 400 mg BPA/kg body weight/day by oral gavage in a 3-day uterotrophic assay, Laws *et al.* (2000) reported uterine wet weight to be significantly increased with doses of 200 and 400 mg BPA/kg. However, the increase in uterine weight was only significant in females necropsied at 6 hours following last dose, and not in females necropsied at 24 hours. The nature of the differences observed between time of necroscopy was not elaborated. In prepubertal female Alpk:AP rats necropsied 24 hours following final oral doses of 400, 600, and 800 mg/kg body weight/day in a 3-day uterotrophic assay, Ashby and Tinwell (1998) reported uterine wet and dry weights to be significantly increased compared to control females receiving arachis oil. Laws *et al.* (2000) reported the *sc* administration of 200 mg BPA resulted in a greater percent increase in uterine weight than with oral administration when necropsied at 6 hours post-final dose, however, the uterine weight of females necropsied at 24 hours post-final *s*c dose was apparently not measured. Furthermore, oral administration of 50, 100, or 400 mg BPA/kg body weight/day to prepubertal females on Days 21 through 35 of age, did not affect body weight or the age at vaginal opening. Ashby and Tinwell (1998) similarly reported that oral administration of up to 800 mg BPA/kg body weight/day in a 3-day uterotrophic assay did not induce premature vaginal opening. Premature vaginal opening was observed, however, in 4 of 7 and 7 of 15 rats administered 600 and 800 mg BPA/kg body weight *sc*, respectively (Ashby and Tinwell, 1998). Regardless, the bioavailability of BPA is dependent upon route of administration and is lower following oral administration (Pottenger *et al.*, 2000) and likely accounts for the greater estrogen-like activity of BPA when administered by *ip* or *sc* injection.

In mice, Papaconstantinou *et al.* (2000) reported on changes in uterine weight, histology and morphometry following the *sc* administration of BPA. In this study, ovariectomized female

B6C3F1 mice were administered BPA in corn oil at doses of 1, 10, 40, 100, and 400 mg/kg body weight/day, or corn oil alone, for 4 days and then terminated 24 hours following final dose. Papaconstantinou *et al.* (2000) reported a significant increase in uterine wet weight at doses ‡ 40 mg BPA/kg body weight/day, with an estimated EC50 of 36 mg/kg body weight/day. In separate studies, Papaconstantinou *et al.* (2000) reported an EC50 of 0.97 g/kg body weight/day for increased uterine weight induced by estradiol. Papaconstantinou *et al.* (2000) indicated the potency of BPA to be approximately 37,000 times lower than estradiol. However, the potency of BPA may actually be lower. As noted by Safe (2000), a direct comparison of potency cannot be made from the estimated EC50 values of Papaconstantinou *et al.* (2000), as the magnitude of uterine weight change induced BPA was >3 times lower than estradiol. Furthermore, the results of Papaconstantinou *et al.* (2000) are somewhat ambiguous in that the inhibition of the estrogenic activity of BPA required a dose of ICI 182,780, an experimental anti-estrogen, that was 10 times greater than the dose required to inhibit the far more potent estradiol. In addition, Papaconstantinou *et al.* (2000) reported that BPA (100 mg/kg body weight/day) significantly increased uterine luminal epithelial height, and the thickness of the stroma and myometrium. These results are ambiguous in that administration of the anti-estrogen ICI 182,780, alone, resulted in significantly increased stromal and myometrial thicknesses similar to that of BPA. Hence, Safe (2000) suggested the effect of BPA on uterine weight reported by Papaconstantinou *et al.* (2000) may be independent of known estrogen receptors. The results of Papaconstantinou *et al.* (2000) are further complicated by apparent age differences of mice between studies separately conducted with either BPA or estradiol.

Rigorous reproductive and developmental toxicity studies with BPA in mice and rats have not demonstrated BPA to be a selective developmental or reproductive toxin. Kwon *et al.* (2000) recently reported that daily oral gavage treatment of pregnant SD rats with BPA had no effect on maternal reproductive parameters or on selected parameters of prepubertal development and reproductive function in F_1 female offspring. Furthermore, exposure to BPA *in utero* and through lactation had no effect on reproductive organ weights or on the micromorphology of the prostrate of F_1 males at 6 months of age. Groups of 8 dams were administered 5 ml corn oil/kg body weight/day as a control, or 3.2, 32, or 320 mg BPA/kg body weight/day, from gestational Day 11 through postnatal Day 20. As a positive reference for estrogenic activity, an additional group of 8 dams was administered 15 g DES/kg body weight/day for a similar duration. Compared to control rats, BPA had no effect on maternal body weights during pregnancy or lactation; liver, kidney, adrenal, ovary, or uterus weights at termination on postnatal Day 21; or the number of live pups per litter. Exposure to BPA did not affect mean pup weights measured on postnatal Days 1 and 7. Kwon *et al.* (2000) measured the volume of the sexually dimorphic preoptic area of the hypothalamus as a morphological marker of sexual differentiation. In female offspring from BPA-treated dams, the mean volume of the sexually dimorphic preoptic nucleus was similar to that of control offspring, as was the age and body weight at vaginal opening, the age at first estrus, the length, number, and duration of estrous cycling, and the lordosis reflex response. In comparison, F_1 females from DES-treated dams demonstrated a significantly greater volume of the sexually dimorphic nucleus of the preoptic area, and a significantly lower number of days in estrus, number of cycles, and length of cycle, as compared to controls. Neither DES or BPA administration to dams had a significant effect on the body weight or reproductive organ weights of male offspring.

The NTP has conducted a complete reproduction and fertility assessment in which CD-1 mice were fed 0, 0.25, 0.5, or 1.0% BPA in the diet. NTP concluded that observed adverse effects on reproductive parameters may be secondary to generalized toxic effects of BPA (NTP, 1985b). Administered within a continuous breeding protocol to 11-week old mice over an 18-week period, doses of 0.5 and 1.0 % BPA in the diet (approximately 875 and 1,750 mg BPA/kg body weight/day) resulted in a significantly lower number of litters per mating pair, number of live pups per litter, and live pup weight. However, F_0 maternal bodyweights were reduced at these doses compared to controls, indicating that the observed effects on litters may be the result of generalized maternal toxicity. A cross-over mating trial with control and 1.0% BPA F_0 mice indicated no adverse effect on mating and fertility, however, a significant reduction in the number of live pups per litter was recorded. The body weight of 1.0% BPA-treated F_0 females was again significantly lower than controls, and histopathological examination of both sexes revealed centrilobular hepatocytomegaly, multifocal necrosis and multinucleated giant hepatocytes in the liver, and variable tubular cell histology in the kidney. Male F_0 mice exposed to 1.0% BPA exhibited a significantly lower seminal vesicle weight and reduced sperm motility, without effects on sperm morphology or concentration. Female F_0 mice exhibited no effects of BPA on reproductive tissues, including the uterus and vaginal mucosa. The continuous exposure of F_1 mice to 0.25, 0.5, or 1.0% BPA in feed, *in utero*, and from birth to approximately 74 days of age, indicated dose-related histopathological effects in the liver and kidneys, consistent with effects reported in F_0 mice and in the NTP carcinogenicity assay described in Section 4.0 (NTP, 1982). Reduced weights of some reproductive organs were observed in male mice; however, histopathological abnormalities were absent. Furthermore, continuous dosing of BPA to F_1 mice had no effects on reproductive function or parameters of the resultant offspring.

Similarly, Morrissey *et al.* (1987) evaluated the developmental toxicity in CD rats and CD-1 mice following oral administration of BPA to pregnant females. Daily doses of 0, 160, 320, or 640 mg BPA/kg body weight/day for rats, or 0, 500, 750, 1,000, or 1,250 mg BPA/kg body weight/day for mice, were administered on Days 6 through 15 of gestation. Females were sacrificed 1 day prior to parturition and fetuses examined for development abnormalities. In mice administered the highest dose of 1,250 mg BPA/kg body weight/day, Morrissey *et al.* (1987) reported a significant increase in percent resorptions per litter, however, effects on maternal body weight and liver weight were also reported at this dose. In rats, Morrissey *et al.* (1987) reported maternal body weights were significantly reduced on gestation Days 11 through 15 at all dose levels of BPA compared to controls; however, no significant effects on fetal development were observed at any dose.

Studies published by Nagel *et al.* (1997), vom Saal *et al.* (1998), and Gupta (2000) contend that adverse effects associated with BPA toxicity may occur at lower doses that are more reflective of estimated human exposure. Specifically, these studies conclude that low doses of BPA may affect prostate growth in male mice. Nagel *et al.* (1997) and vom Saal *et al.* (1998) reported increased prostate and preputial gland weights, decreased epididymal weight and reduced efficiency of sperm production in 6-month old male offspring of female CF-1 dams orally administered 2 or 20 g BPA/kg body weight/day on Days 11 through 17 of gestation. Gupta (2000) reported oral administration of 50 g BPA/kg body weight/day to pregnant CD-1 mice on Days 16 through 18 of gestation significantly increased the anogenital distance and prostate weight of male offspring measured at 3, 21 and 60 days of age. A 2-generation reproduction study of low dose BPA in rats (0.2, 2, 20, and 200 g/kg body weight/day) reported decreased anogenital distances in F_1 and F_2 males and females measured at various times postnatal; however, it was concluded that effects on anogenital distance were not toxicologically relevant considering that no adverse effects of BPA exposure on sexual maturation or reproductive success were observed (CCSRI, 2000). Gupta (2000) also reported epididymis weight was significantly reduced in 60 day old male offspring of BPA-dosed females. The results of Nagel *et al.* (1997), vom Saal *et al.* (1998) and Gupta (2000) are compromised by numerous experimental shortfalls documented by others (Ashby *et al.*, 1999; Cagen *et al.*, 1999a). Furthermore, these results have not been confirmed in more rigorous studies designed specifically to duplicate protocols and validate conclusions (Ashby *et al.*, 1999; Cagen *et al.*, 1999a; Tyl *et al*., 2000).

Ashby *et al.* (1999) and Cagen *et al.* (1999a) undertook independent evaluation of the sexual development of male CF-1 mice exposed to BPA *in utero*, following the protocol of Nagel *et al.* (1997) and vom Saal *et al.* (1998), and thoroughly documenting any exceptions to the protocol. The noted exceptions were specifically included to increase the strength and validity of the results. Ashby *et al.* (1999) concluded that BPA doses of 2 and 20 g BPA/kg body weight/day to pregnant dams did not affect prostate weights or sperm production of resultant male offspring. Ashby *et al.* (1999) further elaborated on data interpretation regarding treatment effects on organ weights and the justification of adjusting for body weight, indicating that differences in body weight may account for observed differences in prostate weights reported by Nagel *et al.* (1997). Cagen *et al.* (1999a) included doses of 0.2 and 200 g BPA/kg body weight/day in addition to the original doses of 2 and 20 g BPA/kg body weight/day employed by Nagel *et al.* (1997) and vom Saal *et al.* (1998). Even with the expanded range of doses tested, no treatment-related effects on reproductive organ weights or sperm production efficiency were observed in 90-day old male offspring of pregnant female mice dosed with BPA on Days 11 through 17 of gestation. Furthermore, histopathological examination of reproductive tissues revealed no adverse effects associated with exposure to low doses of BPA.

A lack of effect of orally administered BPA on the sexual development of rats has also been reported in a well-conducted study by Cagen *et al.* (1999b). In this study, female Wistar rats were administered BPA in drinking water at concentrations of 0, 0.01, 0.1, 1.0, or 10 ppm for 2 weeks prior to mating, and throughout mating (2 weeks), gestation (22 days) and lactation (22 days) periods. At weaning, male rats were selected from litters and maintained, untreated with BPA, until sacrifice at 90 days of age. Cagen *et al.* (1999b) estimated BPA consumption of 1 to 4, 8 to 38, 100 to 391, and 775 to 4,022 g BPA/kg body weight/day for female rats treated with 0.01, 0.1, 1.0, and 10 ppm BPA in drinking water, respectively. Exposure of female rats to BPA throughout mating and gestation did not affect reproductive performance or parameters of the resultant litters. Pathological examination revealed no significant effects of BPA consumption in adult females. In addition to histopathological examination of male offspring, Cagen *et al.* (1999b) further utilized the methodologies reported by Nagel *et al.* (1997) and von Saal *et al.* (1998) who had earlier concluded that there were significant effects of low dose BPA exposure on reproductive development in male mice. In contrast, Cagen *et al.* (1999b) reported that treatment-related effects were not observed on total body, brain, kidney, liver, and reproductive organ weights of male offspring. Furthermore, macro- and microscopic examination of tissues from male offspring indicated that exposure to BPA *in utero* and throughout lactation, through maternal doses of 1 to 4,022 g BPA/kg body weight/day, had no effect on sexual development.

The absence of reproductive toxicity of low doses of BPA is further supported by a recently completed two generation reproduction study in rats (CCSRI, 2000). BPA was administered daily by gavage at doses of 0, 0.2, 2, 20, and 200 g/kg body weight/day to male and female Crj:CD (SD) IGS rats over 2 generations. The duration of exposure for the F_0, F_1 and F_2 generations was approximately 13 weeks for males and 10 weeks for females, 15 weeks for males and 19 weeks for females, and 5 weeks for males and 12 weeks for females, respectively. In summary, BPA had no significant effect on the health or reproductive capabilities of rats at the macro- and microscopic level of examination. Histopathological examination of reproductive tissues did not support adverse toxicological effects of BPA. It was reported that anogenital distances in F_1 and F_2 males and females at all doses of BPA were significantly decreased compared to control rats when measured at various times postnatal; however, it was concluded that effects on anogenital distance were not toxicologically relevant considering sexual maturation and reproductive success were not adversely affected, and given that the recorded differences remained within 5% of the control. It was concluded that daily doses of 0.2, 2, 20, and 200 g BPA/kg body weight/day produced no adverse toxicological effects on F_0, F_1, and F_2 parent rats, had no adverse effect on the reproductive capacity of F_0 and F_1 rats, and had no adverse effect on the development of F_1 and F_2 offspring (CCSRI, 2000).

Moreover, a well-conducted three-generation feeding study with a wide range of BPA doses did not observe adverse effects on reproduction or reproductive development in rats (Tyl *et al*., 2000). Tyl *et al*. (2000) administered BPA to male and female CD rats in the diet *ad libitum* at concentrations of 0, 0.015, 0.3, 4.5, 75, 750, and 7,500 ppm BPA, representing approximate daily doses of 0, 1, 20, 300, 5,000, 50,000, and 500,000 g BPA/kg body weight/day. F_0 males and females were exposed to BPA in the diet (30 animals/sex/dose) continuously through a 10-week pre-breed period, a 2-week mating period, and a 3-week gestation period. F_0 males were killed and necropsied following F_1 birth, while F_0 females were further exposed for 3 weeks until F_1 weaning. F_1 and F_2 offspring (30 animals/sex/dose) were similarly exposed to BPA in the diet prior to mating, and through mating, gestation, and lactation, for a total of 15 to 18 weeks following weaning. A total of 30 F_3 animals/sex/dose were exposed to BPA in the diet until adulthood. Systemic toxicity, reported as decreased body weights and weight gains, was reported in adult rats of each generation at 750 and 7,500 ppm BPA in the diet; however, histopathological effects were not observed at 750 ppm (50,000 g BPA/kg body weight/day). At 7,500 ppm BPA in the diet, Tyl *et al.* (2000) reported a higher incidence of renal tubular degeneration and chronic hepatic inflammation in F_0, F_1, and F_2 adult females. Gross and microscopic examination of reproductive organs of treated rats indicated no adverse effects related to BPA at any dose. In F_1, F_2 and F_3 offspring, a significant reduction in pup body weights, and a delayed acquisition of vaginal patency and preputial separation were reported at 7,500 ppm BPA. Tyl *et al*. (2000) indicated these effects were only observed at the paternally toxic dose of 7,500 ppm (500,000 g BPA/kg body weight/day). A significant increase of 0.03 to 0.04 mm in anogenital distance, at dietary concentrations of 0.015, 0.3, 4.5 and 750 ppm BPA, was only observed in females, and limited to F_2 offspring. Tyl *et al*. (2000) concluded these results were without biological or toxicological significance, and attributed the statistical significance of these values to the precision of the measurement technique employed. Furthermore, exposure to BPA had no effects on the reproductive performance or fertility parameters of both males and females at any dose, in all generations. In conclusion, Tyl *et al*.

(2000) reported an adult systemic toxicity NOAEL of 5,000 g BPA/kg body weight/day and a NOAEL of 50,000 g BPA/kg body weight/day for reproductive and postnatal toxicity.

In summary, *in vitro* and *in vivo* assays demonstrating estrogenic activity are of questionable relevance to assessing the carcinogenicity and reproductive toxicity of BPA, given the absence of endocrine-related tumors or adverse effects on reproductive function reported in well-conducted animal studies. Continuous breeding studies with mice indicate BPA may adversely affect fertility at doses ‡ 875 mg/kg body weight/day; however, paternal toxicity was also observed at these dose levels.

5.4 Genetic and Related Effects

5.4.1 Humans

No data were identified in humans.

5.4.2 Experimental Systems

The genotoxic potential of BPA has been evaluated in numerous assays including both *in vitro* and *in vivo* test systems. The results of the assays are summarized in Table 1.

In Vitro Test Systems

BPA has consistently produced negative results in bacterial reverse mutation tests (Ames tests) completed by different investigators (Haworth *et al.*, 1983; Takahata *et al.*, 1990; Schweikl *et al.*, 1998). In these studies, no increases in mutations, with or without metabolic activation, were observed at any concentration up to cytotoxic levels in any of the *Salmonella typhimurium* strains tested, including strains TA97a, TA98, TA100, TA102, TA1535, and TA1537. Further experiments confirmed these results in *Salmonella typhimurium* TA98, TA100, TA 1535, TA1537, TA 1538 and showed negative results also for *Escherichia coli* strains WP2 and WP2*uvr*A at concentrations up to cytotoxic levels in the absence and presence of metabolic activation (Dean and Brooks, 1978; JETOC, 1996).

The results of standard *in vitro* mammalian genotoxicity studies of BPA also have generally indicated a lack of mutagenic and clastogenic activity. Standard assays include International Conference on Harmonisation (ICH) recognized tests for which OECD testing guidelines are available. BPA did not increase the mutation frequency at the HPRT locus in Chinese hamster

V79 cells in the absence of metabolic activation (Schweikl *et al.*, 1998). Negative results also were obtained in a gene mutation assay in mouse lymphoma L5178Y cells (tk locus) with and without metabolic activation (Myhr and Caspary, 1991). In a second mouse lymphoma L5178Y assay using the microtitre method, BPA was considered to be inconclusive for mutagenic response as a result of conflicting results from the two laboratories involved. Only an assessment (without data) of the mutagenic potential of BPA is given in this paper (Honma *et al.*, 1999). Upon critical evaluation of all data from this trial, Moore *et al.* (1999) concluded that BPA was inconclusive without S9 but negative with S9. Although considered a sensitive assay, the mouse lymphoma assay has been reported to be associated with a high number of false positives (Zeiger *et al.*, 1990). In a non-standard gene mutation assay in Syrian hamster embryo cells, BPA did not increase the mutation frequency at the Na+/K+, ATPase or hprt locus in the absence of metabolic activation (Tsutsui *et al*., 1998; 2000). Experimental difficulties with Syrian hamster embryo cells have limited the routine use of this assay for assessing carcinogenic potential (LeBoeuf *et al.*, 1996).

Negative results for BPA were obtained for the mammalian chromosome aberration test in Chinese hamster ovary (CHO) cells with and without metabolic activation (Ivett *et al*., 1989). In a second chromosome aberration assay, negative results were obtained with S9 up to a maximum scoreable dose (cell count 28% of controls) (Hilliard *et al*., 1998). In this assay, in the absence of S9, a positive response at the two highest doses of BPA was associated with a narrow dose range with steeply increasing cytotoxicity. The same working group (Galloway *et al.*, 1998) reported a further cytogenetic study under the same conditions using only high concentrations of BPA in the absence of S9. Again, positive findings occurred coincident with and are attributed to cytotoxicity due to BPA. BPA did not increase the frequency of chromosomal aberrations in a non-standard chromosome aberration assay with Syrian hamster ovary cells without metabolic activation (Tsutsui *et al.*, 1998). The percentage of aneuploid cells in the near diploid range was increased at BPA concentrations of 50 µM or greater; however, this response was not dose-related. An increase was not observed in the percentage of heteroploid cells with a tetraploid or near-tetraploid number of chromosomes. Furthermore, the finding of aneuploidy in SHE cells was not reproduced under *in vivo* conditions in the mouse micronucleus test (Gudi and Krsmanovic, 1999), a more relevant indicator of genotoxic potential.

In a sister chromatid exchange (SCE) assay in CHO cells, BPA was inactive in the presence of S9 (Ivett *et al*., 1989). In the absence of S9, a small but statistically significant increase in SCE was reported at the highest dose of BPA in the first assay; however, this response was not reproducible at higher doses in a repeat assay.

In an alkaline elution/rat hepatocyte assay for DNA strand breaks, BPA produced dose-related increases in the elution slope, but was scored as negative by the investigators due to extensive cytotoxicity (Storer *et al*., 1996). This assay was reported to be prone to false positive results when loss of membrane integrity was a late event in cell death in relation to induction of endonucleolytic DNA degradation. BPA was inactive (negative) in a transformation assay in BALB/c-3T3 cells (Matthews *et al.*, 1993). In transformation assays with Syrian hamster embryo cells, LeBouef *et al*. (1996) reported a negative response whereas Tsutsui *et al*. (1998) reported a positive but non dose-related response. Non-standard investigations with cell-free systems show that BPA inhibits microtubule polymerization in microtubule proteins from bovine brain (Metzler and Pfeiffer, 1995; Pfeiffer *et al*., 1997), and disrupts the cytoplasmic microtubule complex and mitotic spindle (both reversible), and can induce metaphase arrest (also reversible) and micronuclei in Chinese hamster V79 cells (Pfeiffer *et al*., 1997). However, the formation of micronuclei was accompanied by a reduction of the survival index to 25% demonstrating cytotoxic effects. In addition, the reduction of the survival index to 57.1% induced by the treatment with the vehicle DMSO clearly indicates that the amount of DMSO used in this study was unacceptably high. In another study on V79 cells, some findings like aberrant spindles, increased (tubuline signals and multipolar divisions point to an aneugenic potential of BPA, however, cytotoxicity was not concurrently determined (Ochi, 1999). These results are of questionable significance given that effects were observed at very high concentrations, and also considering that BPA was not genotoxic under *in vivo* conditions in the mouse micronucleus assay (Gudi and Krsmanovic, 1999). In a DNA adduct formation assay employing ^{32}P-postlabelling, 2 main adduct spots were observed in Syrian hamster embryo cells, but were not further characterized (Tsutsui *et al*., 1998). In another DNA adduct formation ^{32}P-postlabelling assay with purified rat DNA, positive responses were attributed to bisphenol o-quinone, an oxidation product of BPA (Atkinson and Roy, 1995a). This quinone derivative (chemically synthesized) formed 6 to 8 adducts after 2 hours incubation with dGMP or DNA. Incubation of BPA with DNA in the presence of a peroxidase activation system and hydrogen peroxide for 3 hours resulted in the formation of 1 major and 7 minor adducts. The chromatographic mobilities of the major adduct and one of the minor adducts formed by BPA in the presence of the peroxidase activation system matched those of 2 adducts formed by bisphenol o-quinone. In subsequent testing, the formation of DNA adducts was inhibited by three known cytochrome P450 inhibitors indicating that activation of bisphenol A to DNA-binding metabolite(s) is P450-dependent. As discussed in the section on mechanistic considerations (Section 5.4.3) bisphenol o-quinone does not appear to be a significant metabolite of BPA under *in vivo* conditions. Metabolism studies suggest that absorbed BPA is rapidly glucuronidated, and not oxidized (Knaak and Sullivan, 1966; Pottenger *et al*., 2000; Nakagawa and Tayama, 2000).

In Vivo Test Systems

Results of a *in vivo* dominant lethal assay in Sprague Dawley male rats, presented in an abstract only, indicated that *ip* injection of BPA at a dose of 85 mg/kg/day for a total of 5 injections was not associated with an increased incidence of dominant lethal findings compared to controls (Bond *et al.*, 1980). The dose tested was indicated to be the maximum tolerated dose. The significance of these results is limited by the lack of details provided.

In a follow-up investigation to their *in vitro* DNA adduct formation study, Atkinson and Roy, (1995b) evaluated the potential for BPA to form DNA adducts *in vivo* following *ip* or oral gavage administration. Groups of four male CD-1 rats were administered *ip* injections of 200 mg BPA/kg and killed at 4, 8, 24, 48, and 72 hours after injection. A vehicle control group (4 male rats) was injected with corn oil. Additionally, groups of 5 male animals were administered BPA by oral gavage at a dose of 200 mg/kg body weight for 4, 8, 12, or 16 days. Another control group was gavaged with corn oil. The authors reported that 2 major and several minor liver DNA adducts were associated with both *ip* and gavage administration of BPA. Further characterization of the 2 major adducts (spots 4 and 6) indicated that they matched the chromatographic adduct profile of bisphenol o-quinone adducts determined *in vitro* (Atkinson and Roy, 1995a). Four hours after single *ip* injection of BPA, levels of adduct for spots 4 and 6 were 2.3 and 5.8 per 10^9 DNA nucleotides. With *ip* injection, the half-life for spot 4 was between 8 and 12 hours; spot 6 was unchanged over the time frame assessed. Conversely, following gavage administration, spot 6 decreased after 8 days (maximum adduct levels were reached after 8 days exposure) with a half-life of approximately 11 days, whereas spot 4 was relatively unchanged. Even though administration of BPA was continued daily for up to 16 days, spot 6 seemed to decrease after 8 days of exposure. The level of adduct for spot 4 was highest after 4 days administration, after which levels decreased slightly and leveled off. An estimation of background DNA adducts associated with corn oil administration was not determinable by autoradiography. The results of the study suggest that, at the high dose administered, BPA may be converted to a metabolite(s), possibly bisphenol o-quinone, capable of DNA adduct formation. The metabolism of BPA in relation to potential for DNA adduct formation is discussed further under Section 5.4.3. The German MAK commission (1996) calculated a covalent binding index (CBI) of 0.01 for BPA. According to Lutz (1979, 1984, 1986), a CBI <0.1 indicates that the substance has no tumorigenic effects, although it has weak DNA binding capacity.

The genotoxic potential of BPA was assessed in a recent *in vivo* mouse micronucleus assay and found to be negative (Gudi and Krsmanovic, 1999). Although unpublished, this study was conducted in compliance with U.S. FDA Good Laboratory Practice Regulations as per 21CFR58, the U.S. EPA GLP Standards, and with the OECD Principles of Good Laboratory Practice. Statements of compliance and quality assurance were included with the report. With this, the results are considered reliable. In this assay, groups of ICR mice (5/sex) were administered BPA *via* oral gavage at doses of 500, 1,000, or 2,000 mg/kg. The doses selected were determined from a range finding study. The negative and positive control groups (5/sex) received corn oil vehicle and cyclophosphamide, respectively. Bone marrow was collected from the mice 24 hours after dose administration. For two additional groups of mice (5/sex), administered the vehicle control or 2,000 mg/kg of BPA, bone marrow was also sampled 48 hours after administration. No mortalities occurred in any of the dose groups. Treatment related clinical signs observed included lethargy and piloerection. The mean ratios of polychromatic erythrocytes to total erythrocytes were reduced in treated groups by 15 to 36% supporting that BPA was bioavailable to the bone marrow. Administration of BPA did not increase the incidence of micronucleated polychromatic erythrocytes per polychromatic erythrocytes at any dose level. A clearly positive response was observed with cyclophosphamide. Also, results for the controls were in keeping with historical control data, thus validating the study. These results indicate that BPA is not genotoxic under the conditions of this assay.

Other Test Systems

Negative responses with BPA were also obtained in the sex-linked recessive lethal test in *Drosophila melanogaster* (Foureman *et al*., 1994) and in an exploratory/non-regulatory gene mutation assay in yeast using *Saccharomyces cerevisiae* strain JD1 (Dean and Brooks, 1978).

5.4.3 Mechanistic Considerations

The few positive *in vitro* findings, generally observed in non-standard assays of BPA, are of questionable significance in light of the large number of standard genotoxicity assays, including the *in vivo* mammalian erythrocyte micronucleus test, that have demonstrated a lack of mutagenic or clastogenic potential for BPA. The results of the standard regulatory assays would be a more reliable indicator of genotoxic potential since the OECD has published detailed protocols for these assays thus allowing for optimum experimental conditions and consistency between laboratories. More importantly, these tests have been validated. In addition, BPA is considered to have no structural alerts for mutagenic activity (Ashby and Tennant, 1988).

With respect to the cell-free assays investigating the aneuploidogenic potential of bisphenol A, the authors cautioned that the inhibitory effect of chemicals on microtubule assembly observed under cell-free conditions may be different in intact cells where metabolism may affect the tubulin binding propensity (Metzler and Pfeiffer, 1995). It also has been suggested that inhibition of microtubule assembly, which can lead to aneuploidy, may be associated with quinone derivatives which can bind to the thiol groups of tubulin (Epe *et al.*, 1990). *In vitro* DNA adduct formation by BPA also has been attributed to bisphenol-o-quinone (Atkinson and Roy, 1995a). However, metabolism studies in rats, as well as results from isolated rat hepatocytes (*in vitro* metabolism), have demonstrated that absorbed BPA would be rapidly converted to bisphenol A-glucuronide, and not the quinone derivative (Knaak and Sullivan, 1966; Pottenger *et al.*, 2000; Nakagawa and Tayama, 2000). The lack of confirmation in the *in vivo* mouse micronucleus assay of the aneugenic effects noted *in vitro* supports this hypothesis.

Hydroxylation (phase I) resulting in quinone formation is generally a minor route for metabolism of phenolic compounds; however, with saturation of the glucuronidation (phase II) capacity, it is possible that some of the BPA may be converted to the quinone. In the *in vivo* DNA adduct study in rats (Atkinson and Roy, 1995b), the 2 major adducts were reported to match the adduct profile of DNA or dGMP-biphenol-o-quinone determined in the *in vitro* study. Only one dose level of 200 mg/kg body weight was tested. In the most recent metabolism study, conducted in rats, the highest dose tested was 100 mg/kg; bisphenol o-quinone was not detected (Pottenger *et al.*, 2000). It is possible that small amounts of bisphenol o-quinone are formed and react with tissue components and therefore are not detected. Although it is plausible that saturation of glucuronidation occurred in the adduct formation study, the lack of overlapping test doses in the metabolism and adduct studies does not allow for a definite determination that the DNA adduct formation test dose saturated the phase II metabolism route. However, given the lack of mutation and clastogenicity observed in the standard genotoxicity assays, the formation of rat hepatic DNA adducts at a high BPA dose is of little relevance for human health given the very low exposures to BPA associated with use of products formulated with this compound.

The overall weight of evidence from mutagenicity/genotoxicity studies, and the lack of a definitive carcinogenic response in animal studies, indicates that BPA is unlikely to be genotoxic to humans.

Table 1 Genetic and Related Effects of Bisphenol A

Test System	Strain/Species	Concentration/ Dose	Findings	Results	Reference
Bacterial Mutation Assays					
Ames test with pre-incubation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537	0, 3.3, 10.0, 33.3, 100.0, 333.3 g/plate (–S9)	No increases in mutations with or without metabolic activation were observed. Three separate laboratories confirmed a lack of mutagenic response under the conditions of this assay.	negative	Haworth *et al.*, 1983
Ames test (standard plate incorporation assay)	*Salmonella typhimurium* strains TA97a, TA98, TA100, TA102	0, 0.05, 0.125, 0.25, 0.50 mg/plate (–S9)	No increases in mutations with or without metabolic activation were observed. Cytotoxicity was observed at the highest concentration.	negative	Schweikl *et al.*, 1998
Ames test	*Salmonella typhimurium* strains TA97, TA98, TA100 and TA102	up to 5 mg/plate (–S9)	No increased in mutations with or without metabolic activation. Cytotoxicity was observed at 0.5 mg/plate for strains TA98 and TA102 and at 1 mg/plate for strains TA97 and TA100.	negative	Takahata *et al.*, 1990
Ames test with pre-incubation	*Salmonella typhimurium* strain TA 1538 and *Escherichia coli* strain WP2 and WP2*uvr*A	up to 1.0 mg/ml (–S9)	In this exploratory test, there were no increases in mutations with or without metabolic activation for any of the strains tested. Cytotoxicity appeared at 0.5 mg/ml and above.	negative	Dean and Brooks, 1978
Ames test	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2*uvr*A	5 µg to 1.25 mg/plate (±S9)	No increases in mutations with or without metabolic activation were observed. Cytotoxicity appeared at 156 µg/plate and above.	negative	JETOC, 1996
In Vitro Mammalian Assays					
Mammalian cell gene mutation assay, HPRT locus	Chinese hamster V79 cells	0.0, 0.1, 0.2 mM (-S9)	No increases in mutation were observed without metabolic activation. At 0.2 mM, a 79% decrease in relative survival was observed. Assay was not tested in the presence of S9.	negative	Schweikl *et al.*, 1998

Table 1 Genetic and Related Effects of Bisphenol A

Test System	Strain/Species	Concentration/ Dose	Findings	Results	Reference
Mammalian cell gene mutation assay, tk$^{+/-}$ locus	Mouse lymphoma L5178Y cells	0, 5, 10, 20, 30, 40, 50, 60 g/ml (-S9; 1st assay); 0, 25, 50, 100, 200 g/ml (+S9); 0, 5, 10, 20, 30, 40, 50 g/ml (-S9; 2 nd assay)	No indication of mutagenicity with or without metabolic activation was observed. Cytotoxicity was observed at concentrations greater than 40 g/ml with and without S9.	negative	Myhr and Caspary, 1991
Mammalian cell gene mutation assay, tk$^{+/-}$ locus (microwell method)	Mouse lymphoma L5178Y cells	not specified (–S9)	Two labs participated in this study. As results were inconsistent between labs (one reported positive results the other negative), BPA was judged to be inconclusive. Only an assessment without data was presented. Other investigators (Moore *et al.*, 1999), upon critical re-evaluation of the data set to more stringent criteria, determined that the results were inconclusive without S9 and negative with S9	inconclusive (-S9); negative (+S9)	Honma *et al.*, 1999; Moore *et al.*, 1999
Mammalian cell gene mutation assay, Na+/K+ ATPase and hprt locus	Syrian hamster embryo cells	25 to 200 μM (no activation system)	No increases in mutations were observed with either locus. Growth was inhibited completely at the highest concentration tested. This is not a standard assay.	negative	Tsutsui *et al.*, 1998; 2000
Chromosome aberration test	Chinese Hamster Ovary (CHO) cells	0, 20, 30, 40 g/ml (-S9) 0, 30, 40, 50 g/ml (1st test, +S9) 0, 30, 40, 50 g/ml (2nd test, +S9)	An increase in aberrations was observed at the highest dose with metabolic activation where cell confluence was reduced by about 70%, but not at this same dose in the repeat assay nor in the assay without S9.	negative	Ivett *et al.*, 1989

Table 1 Genetic and Related Effects of Bisphenol A

Test System	Strain/Species	Concentration/ Dose	Findings	Results	Reference
Chromosome aberration test	CHO cells, clone WBL	0, 350, 400, 450 M (-S9) up to 250 M (+S9	The percentage of cells with chromosome aberrations was significantly increased over negative controls at the two highest concentrations tested without S9. Aberrations were not increased with S9 (up to a maximum scoreable dose; cell count 28% of controls). The positive response was associated with a narrow dose range with steeply increasing cytotoxicity	positive without S9; negative with S9	Hilliard *et al.*, 1998
Chromosome aberration test	CHO cells, clone WBL	400 and 450 µM (-S9)	The percentage of chromosome aberrations was increased in both concentrations, but appeared coincident with cytotoxicity.	positive without S9	Galloway *et al.,* 1998
Chromosome aberration test	Syrian hamster embryo cells	25 to 200 µM (no activation system)	BPA did not increase the frequency of chromosomal aberrations. Growth was inhibited completely at the highest concentration tested. This is not a standard assay.	negative	Tsutsui *et al.*, 1998; 2000
Numerical aberrations assay, aneuploidy, polyploidy	Syrian hamster embryo cells	25 to 200 µM (no activation system)	The percentage of aneuploid cells in the near diploid range were increased at concentrations ‡50 µM; however, this response was not dose related. BPA was not associated with an increase in the percentage of heteroploid cells with a tetraploid or near-tetraploid number of chromosomes. Growth was inhibited completely at the highest concentration tested. This is not a standard assay	inconclusive	Tsutsui *et al.*, 1998; 2000

Table 1 Genetic and Related Effects of Bisphenol A

Test System	Strain/Species	Concentration/ Dose	Findings	Results	Reference
Sister Chromatid Exchange assay	CHO cells	0, 0.8, 2.4, 8.0 g/ml (1^{st} test, -S9) 0, 15, 20, 25 g/ml (2^{nd} test, -S9) 0, 30, 40, 50 g/ml (+S9)	A small, but statistically significant, increase in SCE was observed in the first assay without S9 at 8.0 g/ml; however, this response was not reproducible at higher concentration. BPA did not induce SCE at any concentration up to toxic levels when tested with S9.	negative	Ivett *et al.*, 1989
Alkaline elution/rat hepatocyte assay for DNA strand breaks	Sprague Dawley rat hepatocytes	0, 0.2, 0.3, 0.4, 0.5 mM	BPA produced dose-related increases in the induced elution slope, but was scored as negative (by investigators) due to extensive cytotoxicity. This assay was reported to be prone to false positive results when loss of membrane integrity was a late event in cell death in relation to induction of endonucleolytic DNA degradation.	negative	Storer *et al.*, 1996
BALB/c-3T3 transformation assay	A31-1-13 clone of BABL/c-3T3 cells	indicated only as doses that covered a range of cytotoxic responses of approximately 10 to 100% of the relative cloning efficiency; the average cytotoxic LD_{50} was reported at 0.147 mM (~33.6 g/ml)	A sufficient negative transformation response was obtained in all four experiments conducted. As such, BPA was evaluated as inactive in this transformation assay.	negative	Matthews *et al.*, 1993
Syrian hamster embryo cell transformation assay	Syrian hamster embryo cells	25 to 200 M (no activation system)	Morphological transformation was induced at concentrations ‡ 50 M; however, this response was not dose-related. Growth was inhibited completely at the highest concentration tested.	positive	Tsutsui *et al.*, 1998; 2000

Table 1 Genetic and Related Effects of Bisphenol A

Test System	Strain/Species	Concentration/ Dose	Findings	Results	Reference
Syrian hamster embryo cell transformation assay	Syrian hamster embryo cells	up to 50 g/ml (24-hr exposure); up to 30 g/ml (7-day exposure) (no activation system)	The morphological transformation frequency for BPA was not significantly different from the negative control in either the 24 hour or 7-day exposure assay. The maximum concentrations tested resulted in 44% and 61% relative plating efficiency for 24-hours and 7-day exposure periods, respectively.	negative	LeBoeuf *et al.*, 1996
DNA adduct formation, ^{32}P-postlabelling	Syrian hamster embryo cells	50-200 µM (no activation system)	Two main adduct spots were observed *via* chromatogrpahy at 50 µM and three were observed at ‡ 100 µM. A dose-response trend was observed. The adducts were not further characterized.	positive	Tsutsui *et al.*, 1998
DNA adduct formation, ^{32}P-postlabelling	purified rat DNA	bisphenol o-quinone: 0.75 mg DNA or dGMP: 1 mg BPA: 1 mM peroxidase: 100 g H_2O_2: 1 mM	Bisphenol o-quinone (chemically synthesized), a potential oxidation product of BPA, was reported to form 6-8 DNA adducts. Incubation of BPA with DNA in the presence of peroxidase activation system and hydrogen peroxided also produced 1 major and 7 minor adducts.	positive	Atkinson and Roy, 1995a
Cell-free microtubule polymerization assay	microtubule proteins from bovine brain	10 to 100 M	BPA inhibited microtubule polymerization. Several estrogens were tested in addition to BPA. No correlation was found between estrogen receptor binding affinity and microtubule assembly inhibition. This is not a standard assay.	positive	Metzler and Pfeiffer, 1995
Cell-free microtubule polymerization assay	microtubule proteins from bovine brain	20 to 200 M	An EC_{50} for inhibition of microtubule assembly of 150 g was determined for BPA. This is not a standard assay.	positive	Pfeiffer *et al.*, 1997

Table 1 Genetic and Related Effects of Bisphenol A

Test System	Strain/Species	Concentration/ Dose	Findings	Results	Reference
Micronucleus and microtubule assays for assessing aneuploidogenic potential	Chinese hamster V79 cells	200 M (-S9) (100 M for metaphase arrest assay)	BPA disrupted the cytoplasmic microtubule complex and mitotic spindle (both effects were reversible), induced metaphase arrest (also reversible) and induced micronuclei (CREST-positive) in the V79 cells. These effects suggest aneuploidogenic potential under the conditions of this assay. This is not a standard assay.	positive	Pfeiffer *et al.*, 1997
Microtubule assays for assessing aneuploidogenic potential	Chinese hamster V79 cells	100, 150, 200 µM (-S9)	BPA induced aberrant spindles, increased γ tubuline signals and multipolar divisions which points to an aneugenic potential. This is not a standard assay.	positive	Ochi, 1999
In Vivo Mammalian Assays					
Dominant lethal assay	Sprague Dawley rats, male	0, 85 mg/kg/d intraperitoneally for a total of 5 injections	Abstract only. The treatment dose of 85 mg/kg/d was reported to be the maximum tolerated dose. BPA, at this dose, was not associated with an increased incidence of dominant lethal findings. The significance of these results is limited by the lack of details provided.	negative	Bond *et al.*, 1980
DNA adduct formation, ^{32}P-postlabelling	CD1 male rats	Acute study: 0, 200 mg/kg *via* a single intrapertoneally injection; Subacute study: 0, 200 mg/kg/d *via* gastric gavage for 4, 8, 12 or 16 days.	Two major adducts were associated with both *ip* and gavage administrated of biphenol A. The formation of DNA adducts was inhibited by three known cytochrome P450 inhibitors indicating that activation of BPA to DNA-binding metabolite(s) is P450-dependent.	positive	Atkinson and Roy, 1995b.
Mouse micronucleus assay	ICR mice	0, 500, 1,000, 2,000 mg/kg *via* single oral gavage	BPA was not associated with an increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow cells of male or female mice at 24 or 48 hours after dose administration.	negative	Gudi and Krsmanovic, 1999

Table 1 Genetic and Related Effects of Bisphenol A

Test System	Strain/Species	Concentration/ Dose	Findings	Results	Reference
Other test systems					
Gene mutation assay in yeast	*Saccharomyces cerevisiae* strain JD1	up to 0.5 mg/ml (–S9)	In this exploratory/non-regulatory assay there was no evidence of mutagenicity with or without metabolic activation.	negative	Dean and Brooks, 1978
Sex-linked recessive lethal test	*Drosophila melanogaster*	0, 10000 ppm (feeding)	No mutagenic effects were observed. A mortality rate of 1% in exposed males occurred.	negative	Foureman *et al.*, 1994

6.0 EVALUATION OF BISPHENOL A

Evaluation of the carcinogenic potential of chemical agent is conducted through a weight-of-evidence analysis of all of the available data, including the results of epidemiology, experimental animal, metabolism, genotoxicity, and other mechanistic studies. The relative weight placed on any given result is defined by the quality of the conduct and reporting of the study and of the relevance of the model used to evaluate carcinogenic potential. In this case, data from humans where available are most preferable. Secondarily, carcinogenicity data from long-term studies in animals are used to predict potential human carcinogenicity. This is the case for most chemicals. Other data (*i.e.*, results of genotoxicity, metabolism and mechanistic studies) are used to further critically evaluate and judge the relevance of results obtained from long-term animal studies. Finally, the human exposure to a chemical agent is considered in relation to exposures known to be associated with carcinogenic or precancerous effects from the analyses of the epidemiology and long-term animal studies

For BPA, as with most chemicals, there is no direct evidence of carcinogenic activity in humans. No formal epidemiological analyses of exposures to BPA, in isolation from other chemicals were reported in the scientific literature.

High quality carcinogenicity studies of BPA were available for evaluation from the results of the cancer testing program conducted by the NTP. The results of these studies, one in each of rats and mice, indicate that BPA is without carcinogenic potential. The incidence of tumors reported were generally similar to that of control animals and of types known to spontaneously occur in rodent species. Moreover, any tumor incidence rates (*i.e.*, increased incidence of Leydig cell tumors in male rats, leukemias in high-dose male rats and of leukemias and lymphomas combined in low-dose male mice) which showed a significant association with treatment were not dose-related, reported in only one sex or species, of marginal statistical significance, and within the normal range of incidence values. These factors lead to the conclusion that these findings were not causally related to BPA treatment. This conclusion is supported by the results of other studies including genetic toxicity and metabolism studies.

The results of standardized assays of genetic toxicity demonstrate BPA to be without mutagenic or genotoxic activity *in vivo*. Some studies have reported that BPA disrupts microtubule assembly *in vitro* and is associated with formation of DNA adducts in rat liver following intraperitoneal injection or oral gavage administration at high-doses. These studies, however, are of limited relevance to the assessment of carcinogenic potential of BPA, particularly in light of the negative results of NTP carcinogenicity studies. Moreover, the results of the *in vivo* mouse micronucleus assay, one of the studies validated for evaluating genotoxic potential *in vivo*, demonstrated BPA to be without genotoxic activity.

The metabolic data provide additional support for the conclusion that BPA is non-genotoxic and not carcinogenic. These studies show that orally ingested BPA, following absorption, is metabolized by glucuronidation, a detoxification mechanism, and excreted as the conjugate or in unchanged form in the feces and urine within 24 hours. Very little oxidation, a process which could conceivably produce reactive intermediates (quinones) appears to occur *in vivo*.

BPA has been widely reported to show weak estrogenic activity, however, adverse effects on reproductive function and development have not been established at doses that are not maternally toxic. The NTP 2-year carcinogenicity studies with BPA have not provided indication of tumorigenic activity, proliferative cell growth, or otherwise adverse effects on endocrine-associated tissues. Thus, *in vitro* and *in vivo* assays demonstrating estrogen-like activity are of little relevance to assessing the carcinogenic potential of BPA.

Overall, the available data, including the results of metabolic studies, short-term assays for mutagenic/genotoxic activity, and the long-term toxicity and carcinogenicity studies conducted with rats and mice, were evaluated using a weight-of-evidence approach recommended by IARC and U.S. EPA. It was concluded that BPA is not carcinogenic. The data underpinning this conclusion include:

< The results the NTP studies which provide no substantive evidence to indicate that BPA was carcinogenic.

< The results of short-term tests of genetic toxicity which demonstrate that BPA is without genotoxic or mutagenic activity *in vivo*.

< The metabolic data which do not support the formation of potentially reactive intermediates and, moreover, which demonstrate that BPA is rapidly glucuronidated and excreted.

< The lack of any reports of association between exposure to BPA and human cancer.

< The low level of potential human exposure.

7.0 REFERENCES

ADA. 1998. ADA Council on Scientific Affairs Position Statement: Estrogenic effects of bisphenol A lacking in dental sealants. American Dental Association (ADA). J Greater Houston Dental Soc September: 11.

Ashby, J. and Tennant, R.W. 1988. Chemical structure, Salmonella mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogens among 222 chemicals tested in rodents by the U.S. NCI/NTP. Mutat Res 204:17-115.

Ashby, J. and Tinwell, H. 1998. Uterotrophic activity of bisphenol A in the immature rat. Environ Health Perspect 106:719-720.

Ashby, J., Tinwell, H. and Haseman, J. 1999. Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed *in utero*. Reg Toxicol Pharmacol 30: 156-166.

Atkinson, A. and Roy, D. 1995a. *In vitro* conversion of environmental estrogenic chemical bisphenol A to DNA binding metabolite(s). Biochem Biophys Res Comm 210(2):424-433.

Atkinson, A. and Roy, D. 1995b. *In vivo* DNA adduct formation by bisphenol A. Environ Molec Mutagen 26:60-66.

Bergeron, R. M., Thompson, T. B., Leonard, L. S., Pluta, L. and Gaido, K. W. 1999. Estrogenicity of bisphenol A in a human endometrial carcinoma cell line. Mol Cell Endocrin 150: 179-187.

Bond, G.P., McGinnis, P.M., Cheever, K.L., Harris S.J., Plotnick, H.B., and Niemeier, R.W. 1989. Reproductive effects of bisphenol A. Society of Toxicology 19th Annual Meeting, March 9-13, Washington D.C., Abstracts of papers: A23.

Brotons, J. A., Olea-Serrano, M. F., Villalobos, M., Pedraza, V. and Olea, N. 1995. Xenoestrogens released from laquer coatings in food cans. Environ Health Perspect 103: 608-612.

Cagen, S. Z., Waechter, J. M., Jr., Dimond, S. S., Breslin, W. J., Butala, J. H., Jekat, F. W., Joiner, R. L., Shiotsuka, R. N., Veenstra, G. E. and Harris, L. R. 1999a. Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A. Toxicol Sci 50: 36-44.

Cagen, S. Z., Waechter, J. M., Jr., Dimond, S. S., Breslin, W. J., Butala, J. H., Jekat, F. W., Joiner, R. L., Shiotsuka, R. N., Veenstra, G. E. and Harris, L. R. 1999b. Normal reproductive organ development in Wistar rats exposed to bisphenol A in the drinking water. Reg Toxicol Pharmacol 30: 130-139.

CCSRI. 2000. Two-generation reproduction study of bisphenol A in rats. Chemical Compound Safety Research Institute(CCSRI). Study No.: SR-98101.

Chun, T-Y. And Gorski, J. 2000. High concentrations of bisphenol A induce cell growth and prolactin secretion in an estrogen-responsive pituitary tumor cell line. Toxicol Appl Pharmacol 162:161-165.

Consumer Reports. 2000a. It s only water, right? Consum Rep:(August)17-22.

Consumer Reports. 2000b. Clarifications, corrections. Consum Rep (October)4.

Dean, B.J. and Brooks, T.M. 1978. Toxicity tests with diphenylol propane (DPP): *In vitro* mutations studies. Shell Research Report TLGR.0111.78. Unpublished.

Epe, B., Harttig, U., Stopper, H. and Metzler, M. 1990. Covalent binding of reactive estrogen metabolites to microtubular protein as a possible mechanism of aneuploidy induction and neoplastic cell transformation. Environ Health Perspect 88:123-127.

FDA. 1996. Cumulative exposure estimated for bisphenol A (BPA), individually for adults and infants, from its use in epoxy-based can coatings and polycarbonated (PC) articles. Verbal request of 10-23-95. Memorandum to G. Diachenki, Ph.D., Division of Product Manufacture and Use, HGS-245 from Allan B. Bailey, Ph.D. Chemistry Review Branch, HFS-245. Department of Health and Human Services, Food and Drug Administration.

Foureman, P., Mason, J.M., Valencia, R. and Zimmering, S. 1994. Chemical mutagenesis testing in *Drosophila*. X. Results of 70 coded chemicals tested for the National Toxicology Program. Environ Mol Mutag 23:208-227.

Fung, E. Y. K., Ewoldsen, N. O., St. Germain, H. A., Jr., Marx, D. B., Miaw, C-L., Siew, C., Chou, H-N., Gruninger, S. E. and Meyer, D. M. 2000. Pharmacokinetics of bisphenol A released from a dental sealant. JADA 131:51-58.

Galloway, S.M., Miller, J.E., Armstrong, M.J., Bean, C.L., Skopek, T.R. and Nichols, W.W. 1998. DNA synthesis inhibition as an indirect mechanism of chromosome aberrations: comparison of DNA-reactive and non-DNA-reactive clastogens. Mutation Research 400: 169-186.

General Electric. 1976a. Bisphenol A: Nineteen day oral toxicity study in dogs. Unpublished report of General Electric (IRDC study 313-079).

General Electric. 1976b. Reproductive and ninety day oral toxicity study in rats. Unpublished report of General Electric (IRDC study 313-078).

Gudi, R. and Krsmanovic, L. 1999. Mammalian erythrocyte micronucleus test. BioReliance. AA12WJ.123.BTL.

Gupta, C. 2000. Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. P.S.E.B.M. 224: 61-68.

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W. and Zeiger, E. 1983. Salmonella mutagenicity test results for 250 chemicals. Environ Mutagen Suppl 1:3-142.

Hilliard, C.A., Armstrong, M.J., Bradt, C.I., Hill, R.B., Greenwood, S.K. and Galloway, S.M. 1998. Chromosome aberrations *in vitro* related to cytotoxicity of nonmutagenic chemicals and metabolic poisons. Environ Molec Mutag 31:316-326.

Honma, M., Hayashi, M., Shimada, H., Tanaka, N., Wakuri, S., Awogi, T., Yamamoto, K.I., Kodani, N.U., Nishi, Y., Nakadate, M., and Sofuni, T. 1999. Evaluation of the mouse lymphoma tk assay (microwell method) as an alternative to the *in vitro* chromosomal aberration test. Mutagenesis 14(1):5-22.

Howe, S. R. and Borodinsky, L. 1998. Potential exposure to bisphenol A from food-contact use of polycarbonate resins. Food Addit Contamin 15: 370-375.

Howe, S. R., Borodinsky, L. and Lyon, L. S. 1998. Potential exposure to bisphenol A from food-contact use of epoxy can coatings. J Coatings Tech 70: 69-74.

Ivett, J.L., Brown, B.M., Rodgers, C., Anderson, B.E., Resnick, M.A. and Zeiger, E. 1989. Chromosomal aberrations and sister chromatid exchange tests in Chinese Hamster Ovary cells *in Vitro*. IV. Results with 15 chemicals. Environ Mol Mutagen 14:165-187.

JETOC. 1996. Mutagenicity test data of existing chemical substances. Japan Chemical Industry Ecology-Toxicology & Information Center, January 1996, Japan

Kawamura, Y., Koyama, Y., Takeda, Y. and Yamada, T. 1998. Migration of bisphenol A from poly-carbonate products. J Food Hygiene 99: 206-212.

Knaak, J.B., and Sullivan, L.J. 1966. Metabolism of bisphenol A in the rat. Toxicol Appl Pharmacol 8(2):175-184.

Kwon, S., Stedman, D. B., Elswick, B. A., Cattley, R. C. and Welsch, F. 2000. Pubertal development and reproductive functions of Crl:CD BR Sprague-Dawley rats exposed to bisphenol A during prenatal and postnatal development. Toxicol Sci 55:399-406.

Laws, S. C., Carey, S. A., Ferrell, J. M., Bodman, G. J. and Cooper, R. L. 2000. Estrogenic activity of octylphenol, nonylphenol, bisphenol A and methoxychlor in rats. Toxicol Sci 54: 154-167.

LeBoeuf, R.A., Kerckaert, G.A., Aardema, M.J., Gibson, D.P., Brauninger, R. and Isfort, R.J. 1996. The pH 6.7 Syrian hamster embryo cell transformation assay for assessing the carcinogenic potential of chemicals. Mutat Res 356:85-127.

Lutz, W.K. 1979. In vivo covalent binding of organic chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis. Mutat Res 65:289-356.

Lutz, W.K. 1984. Structural characteristics of compounds that can be activated to chemically reactive metabolites: use for a prediction of carcinogenic potential. Arch Toxicol Suppl 7:194-207.

Lutz, W.K. 1986. Quantitative evaluation of DNA binding data for risk estimation and for classification of direct and indirect carcinogens. J Cancer Res Clin Oncol 112:85-91.

MAK, Critical data evaluation for MAK values and classification of carcinogens. 1996. Volume 13. WILEY-VCH Weinhein, New York, Chichester, Brisbane, Singapore, Toronto.

Matthews, E.J., Spalding, J.W. and Tennant, R.W. 1993. Transformation of BALB/c-3T3 cells: V. Transformation responses of 168 chemicals compared with mutagenicity in Salmonella and carcinogenicity in rodent bioassays. Environ Health Perspect Suppl 101 (Suppl. 2):347-482.

Metzler, M. and Pfeiffer, E. 1995. Effects of estrogens on microtubule polymerization *in Vitro*: Correlation with estrogenicity. Environ Health Perspect 103 (Suppl. 7):21-22.

Moore, M.M., Harrington-Brock, K. and Cole, J. 1999. Issues for conducting the microtitre version of the mouse lymphoma thymidine kinase (tk) assay and a critical review of data generated in a collaborative trial using the microtitre method. Mutagenesis 14(3):271-281.

Morrissey, R. E., George, J. D., Price, C. J., Tyl, R. W., Marr, M. C. and Kimmell, C. A. 1987. The developmental toxicity of bisphenol A in rats and mice. Fundam Appl Toxicol 8:571-582.

Myhr, B.C. and Caspary, W.J. 1991. Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: Results for 31 coded compounds in the national toxicology program. Environ Molec Mutag 18:51-83.

Nagel, S. C., vom Saal, F. S., Thayer, K. A., Dhar, M. G., Boechler, M. and Welshons, W. V. 1997. Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative *in vivo* bioactivity of the xenoestrogens bisphenol A and octylphenol. Environ Health Perspect 105: 70-76.

Nakagawa, Y. and Tayama, S. 2000. Metabolism and cytotoxicity of bisphenol A and other bisphenols in isolated rat hepatocytes. Arch Toxicol 74(2):99-105.

NTP. 1982. NTP Technical Report on the Carcinogenesis Bioassay of Bisphenol A in F344 Rats and $B6C3F_1$ Mice (Feed Study). National Toxicology Program. US. Department of Health and Human Services. Public Health Service. National Institute of Health.

NTP. 1985a. Teratologic evaluation of bisphenol A (CAS No. 80-05-7) administered to CD-1 mice on gestational days 6 through 15. Report NTP-85-088; NTIS PB85-205102.

NTP. 1985b. Final Report. Bisphenol A: Reproduction and fertility assessment in CD-1 mice when administered in the feed. National Toxicology Program. NTP-85-192. NTIS PB86103207.

Ochi, T. 1999. Induction of multiple microtubule-organizing centers, multipolar spindles and multipolar division in cultured V79 cells exposed to diethylstilbestrol, estradiol-17b and bisphenol A. Mutation Research 431:105-121.

Olea, N., Pulgar, R., P rez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A. N. and Sonnenschein, C. 1996. Estrogenicity of resin-based composites and sealants used in dentistry. Environ Health Perspect 104:298-305.

Papaconstantinou, A. D., Umbreit, T. H., Fisher, B. R., Goering, P. L., Lappas, N. T. and Brown, K. M. 2000. Bisphenol A-induced increase in uterine weight and alterations in uterine morphology in ovariectomized B6C3F1 mice: Role of the estrogen receptor. Toxicol Sci 56: 332-339.

Pfeiffer, E., Rosenberg, B., Deuschel, S. and Metzler, M. 1997. Interference with microtubules and induction of micronuclei *in vitro* by various bisphenols. Mutat Res 390:21-31.

Pottenger, L. H., Domoradzki, J. Y., Markham, D.A., Hansen, S. C., Cagen, S. Z. and Waechter, J. M., Jr. 2000. The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. Toxicol Sci 54: 3-18.

Pulgar, R., Olea-Serrano, M. F., Novillo-Fertrell, A., Rivas, A., Pazos, P., Pedraza, V., Navajas, J-M. And Olea, N. 2000. Determination of bisphenol A and related aromatic compounds released from bis-GMA-based composites and sealants by high performance liquid chromatography. Environ Health Perspect 108: 21-27.

Safe, S. 2000. Bisphenol A and related endocrine disruptors. Toxicol Sci 56: 251-252.

Schweikl, H., Schmalz, G. and Rackebrandt, K. 1998. The mutagenic activity of unpolymerized resin monomers in *Salmonella typhimurium* and V79 cells. Mutat Res 415:119-130.

Steinmetz, R., Brown, N. G., Allen, D. L., Bigsby, R. M. and Ben-Jonathon, N. 1997. The environmental estrogen bisphenol A stimulates prolactin release *in vitro* and *in vivo*. Endocrinology 138: 1780-1786.

Steimetz, R., Mitchner, N. A., Grant, A., Allen, D. L., Bigsby, R. M., and Ben-Jonathan, N. 1998. The xenoestrogen bisphenol A induces growth, differentiation, and *c-fos* gene expression in the female reproductive tract. Endocrinology 139: 2741-2747.

Storer, R.D., McKelvey, T.W., Kraynak, A.R., Elia, M.C., Barnum, J.E., Harmon, L.S., Nichols, W.W., and DeLuca, J.G. 1996. Revalidation of the in vitro alkaline elution/rat hepatocyte assay for DNA damage: improved criteria for assessment of cytotoxicity and genotoxicity and results for 81 compounds. Mutat Res 368:59-101.

Takahashi, O. and Oishi, S. 2000. Disposition of orally administered 2,2-bis(4-hydroxyphenyl)propane (bisphenol A) in pregnant rats and the placental transfer to fetuses. Environ Health Perspect 108:931-935.

Takahata, J., Tamakawa, K., Takahashi, Y., Seki, T., Tsunoda, A., Nohmi, T., and Sofuni, T. 1990. Mutagenicity of environmental chemicals II, bisphenol A. Sendai-shi Eisei Kenkyushoho (Japan) 20:245-247.

Til, H.P., Roverts, W.G., and Beems, R.B. 1978. Sub-chronic (90 day) oral toxicity study with diphenylolpropane (DPP) in rats. Unpublished report (No. R 6229) of TNO, Holland.

Tsutsui, T., Tamura, Y., Yagi, E., Hasegawa, K., Takahashi, M., Maizumi, N., Yamaguchi, F., and Barrett, J. Carl. 1998. Bisphenol-A induces cellular transformation, aneuploidy, and DNA adduct formation in cultured syrian hamster embryo cells. Int J Cancer 75:290-294.

Tsutsui, T., Tamura, Y., Suzuki, A., Hirose, Y., Kobayashi, M., Nishimura, H., Metzler, M., Barrett, J.C. 2000. Mammalian cell transformation and aneuploidy induced by five bisphenols. Int J Cancer 86:151-154.

Tyl, R.W., Myers, C.B., and Marr, B.A. 2000. Three-generation reproductive toxicity evaluation for bisphenol A administered in the feed to CD (Sprague-Dawley) rats. Unpublished report (65C-07036-000) of Research Triangle Institute, North Carolina.

U.S. EPA. 1993. Bisphenol A. United States Environmental Protection Agency (U.S. EPA). IRIS database. http://www.epa.gov/ngispgm3/iris/subst/0356.htm.

U.S. EPA. 1998. Endocrine Disruptor Screening Program; Proposed Statement of Policy. United States Environment Protection Agency. Fed Reg (U.S.) 63(248):71541-71568.

Villalobos, M., Olea, N., Brotons, J. A., Olea-Serrano, M. F., de Almodovar, J. M. R. and Pedraza, V. 1995. The E-screen assay: A comparison of different MCF7 cell stocks. Environ Health Perspect 103: 844-850.

vom Saal, F. S., Cooke, P. S., Buchanan, D. L., Palanza, P., Thayer, K., Nagel, S. C., Parmigiani, S. and Welshons, W. V. 1998. A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production and behavior. Toxicol Indust Health 14: 239-260.

Yoon, K., Pellaroni, L., Ramamoorthy, K., Gaido, K. and Safe, S. 2000. Ligand structure-dependent differences in activation of estrogen receptor _ in human HepG2 liver and U2 osteogenic cancer cell lines. Mol Cell Endocrin 162: 211-220.

Zacharewski, T. 1998. Identification and assessment of endocrine disruptors: Limitations of *in vitro* and *in vivo* assays. Environ Health Perspect 106(Suppl 2):577-582.

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H. and Tennant, R.W. 1990. Evaluation of four *in vitro* genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. Environ Molecul Mutagen 16 (Suppl. 18):1-14.